Appendix B

Chemical Research in Toxicology

Perspective

pubs.acs.org/crt

Oral Cell DNA Adducts as Potential Biomarkers for Lung Cancer Susceptibility in Cigarette Smokers

Stephen S. Hecht*

Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota 55455, United States

ABSTRACT: This perspective considers the use of oral cell DNA adducts, together with exposure and genetic information, to potentially identify those cigarette smokers at highest risk for lung cancer, so that appropriate preventive measures could be initiated at a relatively young age before too much damage has been done. There are now well established and validated analytical methods for the quantitation of urinary and serum metabolites of tobacco smoke toxicants and carcinogens. These metabolites provide a profile of exposure and in some cases lung cancer risk, but they do not yield information on the critical DNA damage parameter that leads to mutations in cancer growth control genes such as *KRAS* and *TP53*. Studies demonstrate a correlation between changes in the oral cavity and lung in cigarette smokers, due to the field effect of tobacco smoke. Oral cell DNA is readily obtained in contrast to DNA samples from the lung. Studies in which oral cell DNA and salivary DNA have been analyzed for specific DNA adducts are reviewed; some of the adducts identified have also been previously reported in lung DNA from smokers. The multiple challenges of developing a panel of oral cell DNA adducts that could be routinely quantified by mass spectrometry are discussed.

CONTENTS

INTRODUCTION

The tobacco control community has made great strides in the past several decades to denormalize the use of tobacco products, especially combusted ones such as cigarettes, cigars, and pipes. Smoking has become, in part, a socioeconomic phenomenon, so most North American and Western European readers of this perspective will never encounter cigarette smokers in indoor environments and probably rarely in the outdoors during their normal daily routine. They may not realize that in spite of this remarkable progress there are still about 40 million adult smokers in the U.S. and an astounding one billion in the world.[1,2] It is likely that many of these smokers are generally aware of the hazards of cigarette smoking but that they cannot break their habit because nicotine, the principal alkaloid of the tobacco plant, is highly addictive.[3]

Cigarette smoking is the major cause of lung cancer, a disease that is generally detected too late for effective curative therapy. While some remarkable advances in lung cancer therapy have been achieved, the overall 5 year survival rate varies from 4–17% depending on stage and regional differences, and median survival following diagnosis is typically measured in months.[4] Cigarette smoking causes 90% of lung cancer in the U.S., where 158,080 deaths were expected in 2016, making it the leading cause of cancer death in both men and women.[5] Worldwide, there were 1,589,000 deaths from lung cancer in 2012, an average of about 3 per minute.[6] Cigarette smoking caused 80% of this worldwide death toll in males and 50% in females.[7] This is an immense public health crisis, but it receives relatively little attention because it is so common.

While cigarette smoking is clearly the major cause of lung cancer, only 11% of female and 24% of male lifetime smokers will get lung cancer by age 85 or greater, and this relatively

Special Issue: CRT30

Received: October 7, 2016
Published: November 16, 2016

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

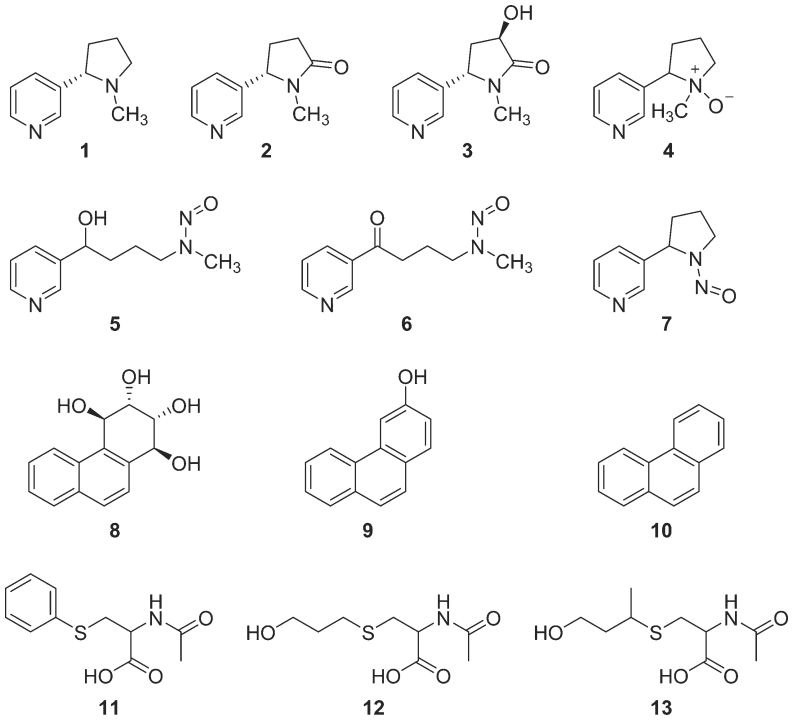


Figure 1. Structures of compounds discussed in the text with respect to quantitation of parent compounds and metabolites in the urine of people who use tobacco products.

Table 1. Panel of Tobacco Smoke Carcinogen and Toxicant Metabolites Quantified in Urine

urinary metabolite or parent compound	biological effect(s) represented	advantages	risk biomarker in smokers?	disadvantages	refs
total nicotine equivalents	toxicity/addiction	measures nearly 90% of the nicotine dose; relatively specific to tobacco use	yes (Shanghai and Singapore cohorts)	not a direct measure of DNA damage	73
total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	lung cancer	good measure of lung carcinogen dose (NNK); specific to tobacco use	yes (Shanghai and Singapore cohorts; PLCO − serum)	not a direct measure of DNA damage	65,74−76
phenanthrene tetraol (PheT)	cancer	good measure of polycyclic aromatic hydrocarbon (PAH) dose plus metabolic activation	yes (Shanghai cohort)	not a direct measure of DNA damage	65,75
3-hydroxyphenanthrene (3-OH-Phe)	cancer	good measure of PAH exposure	not known	not a direct measure of DNA damage or metabolic activation	77
S-phenylmercapturic acid (SPMA)	cancer	good measure of benzene exposure	no	confounded by *GST-T1* status	78,79
3-hydroxypropylmercapturic acid (3-HPMA)	toxicity, cancer(?)	good measure of acrolein exposure	no	not a direct measure of DNA damage	66
3-hydroxy-1-methylpropylmercapturic acid (HMPMA)	toxicity, cancer(?)	good measure of crotonaldehyde exposure	no	not a direct measure of DNA damage	66
monohydroxybutylmercapturic acid (MHBMA)	cancer	good measure of 1,3-butadiene exposure	not known	confounded by *GST-T1* status	78,80
F_2-isoprostanes (8-*iso*-PGF2α)	oxidative damage	accepted measure of oxidative damage	not known	not a direct measure of DNA damage	81
prostaglandin E2 metabolite (PGEM)	inflammation	biomarker of inflammation	not known	not a direct measure of DNA damage	82

small percentage is not due to competing causes of death from smoking.[8] The major goal of the research approach discussed in this perspective is to identify individuals who are highly susceptible to the carcinogenic effects of tobacco products. These individuals would be candidates for intensive lung cancer surveillance and screening, increasing the probability of detection of a tumor at an early stage. When lung cancer is detected in its early stages, surgical resection can achieve far higher 5 year survival rates, 50−70%, than when it is detected at later stages. In this perspective, we are not proposing methods for early detection of tumors such as the identification in exhaled breath condensate of specific proteins which are characteristic of lung tumors[9] but rather identification of susceptible individuals. While there are already algorithms relating various parameters to lung cancer susceptibility, they are mostly retrospective in nature, with pack-years of cigarette smoking being a major prognostic factor.[10−15] Thus, these algorithms are typically applied to subjects who are older, when

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

the process may be more advanced.[16−20] Our ultimate goal is to develop a risk model that is prospective in nature. Overall, there would be a greater probability of success if one identifies high risk individuals early in the carcinogenic process. Even if this were effective in only 10% of tobacco users, the outcome could be prevention of more than 15,000 lung cancer deaths per year in the U.S. alone and massive savings in terms of dollars not spent on therapy.

URINARY AND SERUM METABOLITES AS BIOMARKERS OF LUNG CANCER RISK

In our ongoing research to date, we have focused on several biomarkers of tobacco smoke toxicant and carcinogen uptake as quantified by the relevant parent substances and metabolites detected in urine.[21] Thus, we and others have developed and applied analytically validated methods for urinary substances such as total nicotine equivalents (the sum of nicotine (**1**), cotinine (**2**), 3′-hydroxycotinine (**3**), and their glucuronides, as well as nicotine-*N*-oxide (**4**)); total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, **5**), a metabolite of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, **6**); total *N*′-nitrosonornicotine (NNN, **7**), an important tobacco-specific carcinogen; *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT, **8**) and 3-hydroxyphenanthrene (**9**), metabolites of the representative polycyclic aromatic hydrocarbon phenanthrene (**10**); *S*-phenylmercapturic acid (SPMA, **11**), a metabolite of the carcinogen benzene; 3-hydroxypropylmercapturic acid (HPMA, **12**), a metabolite of acrolein; and 3-hydroxy-1-methylpropylmercapturic acid (HMPMA, **13**), a metabolite of crotonaldehyde (see Figure 1 for structures).[22−25] Massive amounts of data demonstrate the involvement of these classes of compounds in cancer induction by tobacco smoke.[3,8] Some advantages and disadvantages of selected urinary biomarkers are summarized in Table 1.

We have collaborated with epidemiologists to evaluate the relationship of these urinary metabolites to cancer, as determined in prospective epidemiology studies. These studies collect and store biosamples such as blood or urine from large numbers of healthy subjects, then follow the subjects for decades until sufficient numbers of cancer cases occur for statistical analysis. Samples from the cases and matched controls without cancer are recovered from freezers and analyzed for specific biomarkers. This is the most powerful epidemiologic study design because the subjects are recruited and interviewed when healthy, so the eventual occurrence of disease has no bearing on their biomarker levels. The results of these studies have been reviewed.[26,27] In summary, statistically significant relationships of urinary total cotinine (**2** plus its glucuronide), total NNAL (**5**), and PheT (**8**) with lung cancer risk, and total NNN (**7**) with esophageal cancer risk were observed in prospective epidemiological studies of male smokers in Shanghai. Urinary total cotinine and total NNAL were related to lung cancer risk in a study of male and female smokers in Singapore, and total NNAL in serum was related to lung cancer risk in a study of male and female smokers in the U.S.[26,27] Levels of urinary SPMA (**11**), HPMA (**12**), and HMPMA (**13**) were not independently related to lung cancer (after correction for cotinine levels) in the Shanghai study. These results support the use of total cotinine, total NNAL, and PheT as possible biomarkers of lung cancer risk, but the relationships uncovered to date, when examined in the context of receiver operating characteristics analysis, are not strong enough to support their independent application as predictive biomarkers.

SPECIFIC DNA ADDUCTS AS BIOMARKERS OF LUNG CANCER RISK

The urinary and serum metabolites discussed above are mainly biomarkers of exposure and do not inform us about the critical parameter: DNA adduct levels. Thus, an individual with high exposure but excellent detoxification or DNA repair capacity will not have relatively high DNA adduct levels and would be misclassified as having high risk based on exposure measurements only. Conversely, an individual with relatively low exposure but high levels of metabolic activation or low DNA repair capacity would be classified as low risk based on exposure biomarkers only. Therefore, the next logical step in the development of a predictive biomarker formula is to quantify DNA adducts. In this perspective, we discuss DNA adducts in human oral cells, quantified by mass spectrometry, as potentially useful biomarkers for lung cancer risk. Our main hypothesis is that these DNA adduct measurements in combination with certain exposure biomarkers as well as selected genetic information will produce a predictive algorithm for susceptibility to lung cancer in smokers.

The oral mucosa is the mucous membrane lining the inside of the mouth. Oral mucosa cells are an excellent source of material for evaluating DNA adducts and molecular changes potentially related to cancer.[28−31] Collection of oral mucosa cells is relatively simple, in contrast to bronchial brushings and sputum, which can be difficult, expensive, and impractical to obtain. Multiple studies have demonstrated similarities in molecular changes between oral mucosa cells and bronchial cells obtained from the same individuals, particularly in smokers, consistent with the field carcinogenesis concept of lung and upper aerodigestive tract cancer.[32−38] In one large study, a strong association was found between promoter methylation patterns of the *p16* and *FHIT* genes in oral tissues and bronchial brush specimens from smokers.[32] In another study, similarities were found in gene expression changes in the oral and bronchial mucosa, and smoking altered the expression of numerous genes compared to observations in never smokers.[35] A third study demonstrated that smoking-induced gene expression changes in the bronchial airway were reflected both in the nasal and buccal epithelium.[34] Collectively, these results support the use of oral mucosa cells as a surrogate for DNA adduct formation in the lung.[38,39]

Why not quantify DNA adducts directly in lung tissue? As noted above, bronchial brushings and sputum can be difficult to obtain. However, there have been many studies of DNA adduct levels in lung tissue from smokers, mainly obtained during surgery for lung cancer or other diseases. These studies have been reviewed.[40−43] Multiple investigations using nonspecific DNA adduct detection methods such as ^{32}P-postlabeling and immunoassay demonstrate higher levels of DNA adducts of unknown structure in lung tissue from smokers than from nonsmokers. The characterization of the DNA adducts frequently seen in ^{32}P-postlabeling studies and often elevated in smokers' lung tissue typically described as "a diagonal radioactive zone", remains a challenge. This material was originally thought to be derived from polycyclic aromatic hydrocarbons, but studies by Arif et al. do not support that assignment.[44] Relatively few structurally specific DNA adducts have been characterized in lung tissue.[45] These are summarized in Figure 2, the content of which is from a review published in

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

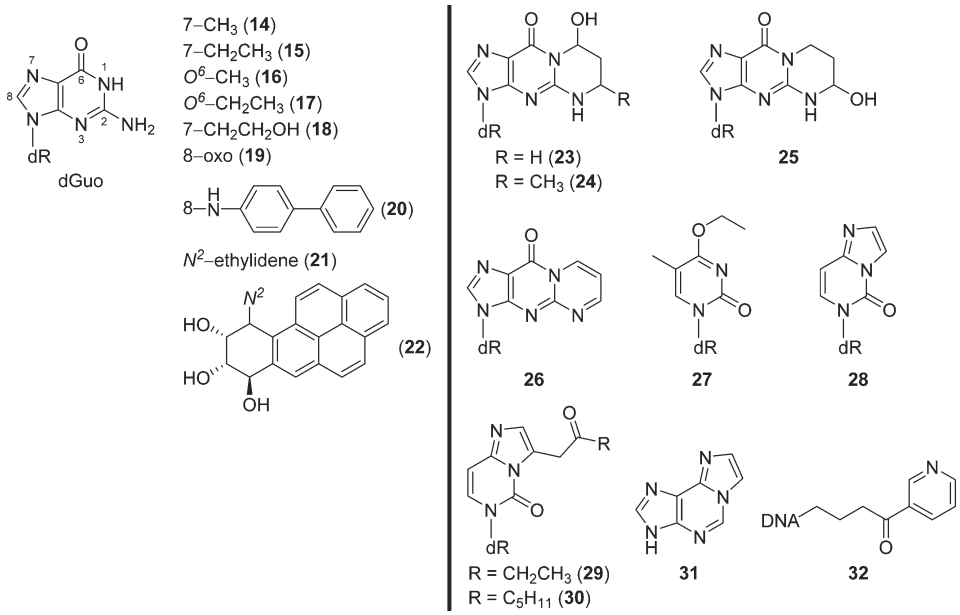


Figure 2. Structures of DNA adducts reported in human lung tissue, using structure-specific methods. Monosubstituted dGuo adducts are to the left of the vertical line and all others to the right. The following DNA adducts have also been detected in DNA from human oral tissue or saliva: **15**, **19**, **20**, **21**, **23**, **24**, **27**, **28**, **31**, and **32**. Adapted with permission from ref 42. Copyright 2012 John Wiley and Sons.

2012;[42] there have been no new characterizations since then. Detected adducts include 7- and O^6-alkylguanines such as **14**–**17**, aromatic-substituted guanines such as **20** and **22**, and a variety of cyclic and etheno-type adducts such as **23**–**26** and **28**–**31**, as well as the HPB releasing adducts **32**. Most of the DNA adducts illustrated in Figure 2 were detected in relatively small studies, and some of them such as the benzo[*a*]pyrene adduct **22** were frequently not detected.[46] None have been unequivocally related to lung cancer risk. Even the sources and identities of some of these DNA adducts are in many cases unclear. For example, the simple 7-alkylguanine, O^6-alkylguanine, and O^4-ethylthymidine adducts could result from direct acting methylating or ethylating agents in cigarette smoke, which are mainly uncharacterized, or from the very small amounts of dialkylnitrosamines present in smoke.

DNA ADDUCTS IN HUMAN ORAL TISSUE AND SALIVA

Earlier studies on DNA adducts in oral tissue, detected exclusively by nonspecific immunoassay and ^{32}P-postlabeling assays, have been reviewed.[28] More recent studies which used chemically specific assays, mainly but not exclusively based on mass spectrometry, are summarized here.

Borthakur et al. quantified 8-oxo-dGuo (**19**, Figure 2) in buccal mucosa cells as a marker of oxidative damage. Subjects were nonsmokers who reported being healthy and not drinking alcohol excessively. The subjects gave blood and buccal cells at baseline and after 3 and 7 days. The participants rinsed their mouth with distilled water before collection of buccal cells using a soft bristle toothbrush. Levels of 8-oxo-dGuo were quantified by HPLC with electrochemical detection. The results demonstrated that 8-oxo-dGuo/dGuo levels were relatively stable over a 7 day period and were 3–4 times higher in buccal cell DNA (30–40 adducts per 10^6 dGuo) than in leukocyte DNA.[31]

Bessette et al.[47] screened for DNA adducts in buccal cell DNA of cigarette smokers using data-dependent constant neutral loss MS^3 in which the neutral loss of the deoxyribose moiety in the MS/MS scan triggers the acquisition of MS^3 product ion spectra of the aglycone. The study was initiated by spiking buccal cell DNA samples with several authentic DNA adducts derived from acrolein, 4-hydroxynonenal, 4-aminobiphenyl, and benzo[*a*]pyrene, and from metabolically activated forms of the heterocyclic aromatic amines 2-amino-3,8-dimethyl-3*H*-imidazo[4,5-*f*]quinoxaline (methyl-IQx), 2-amino-α-carboline, and 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP), at a level of 5 adducts per 10^8 bases. All of the adducts spiked into the buccal cell DNA were clearly detected. However, in unspiked buccal cell DNA samples, only adducts **23** and **25** (Figure 2), derived from acrolein, were unequivocally detected. The MS response for these adducts in the unspiked samples were 10–50 times greater than the response of the other adducts. The levels of the acrolein adducts were estimated to be above 5 adducts per 10^7 DNA bases. These levels were quite similar to those first reported more than 10 years earlier in gingival tissue of cigarette smokers using ^{32}P-postlabeling.[48] That study provided convincing evidence based on HPLC analysis for the presence in gingival tissue of smokers of acrolein adduct **23** and both stereoisomers of the crotonaldehyde adduct **24** (Figure 2). Levels of all of these adducts were significantly higher in smokers than in nonsmokers. Further studies by this group have investigated the use of monoclonal antibodies and immunohistochemistry for analysis of the acrolein-DNA adducts in human oral cells.[49,50]

Further evidence for the presence of DNA adducts of acrolein and crotonaldehyde in oral samples was provided by Chen and Lin who used a validated nanoflow-LC-nanospray ionization tandem MS method to quantify these adducts in 27 human salivary DNA samples from healthy volunteers.[51] They found mean adduct levels of 104 ± 50 adducts/10^8 nucleotides for acrolein adducts (consisting mostly of **23**, Figure 2) and 7.5 adducts/10^8 nucleotides for **24**. They also used this method to quantify the etheno adducts **28** and **31** (Figure 2) as well as 1,N^2-ethenodGuo (**33**, Figure 3), with average levels of 99 ±

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367–375

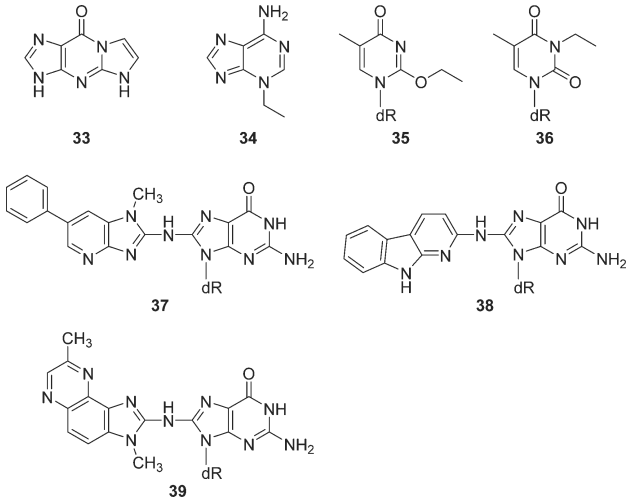


Figure 3. Structures of DNA adducts reported in human saliva but not in the human lung.

50, 72 ± 49, and 391 ± 198 adducts/10^8 nucleotides, respectively. These three adducts were highly correlated with each other suggesting a common source, most likely lipid peroxidation.

The Chen group also used nanoflow-LC-nanospray ionization tandem MS to quantify several other DNA adducts in human saliva.[52,53] Neutral thermal hydrolysis of salivary DNA released 3-Et-Ade (**34**, Figure 3) and 7-Et-Gua (**15**, Figure 2). The mean levels of 3-Et-Ade in 15 smokers and 15 nonsmokers were 12.6 ± 7.0 and 9.7 ± 5.3 per 10^8 normal nucleotides, respectively, while those of 7-Et-Gua were 14.1 ± 8.2 and 3.8 ± 2.8 per 10^8 normal nucleotides.[52] Further studies focused on thymidine adducts. Starting with 50 μg of DNA isolated from 3.5 mL of saliva, they were able to detect O^2-Et-dThd (**35**, Figure 3), N^3-Et-dThd (**36**, Figure 3), and O^4-Et-dThd (**27**, Figure 2) in saliva DNA samples from 20 smokers, with levels ranging from 4 to 5 adducts per 10^8 normal nucleotides, while these adducts were nondetectable in saliva samples from nonsmokers.[53]

Saliva samples from 37 human volunteers on unrestricted diets were analyzed for DNA adducts of heterocyclic aromatic amines and related compounds by LC-MS/MS^n by Bessette et al.[54] The dGuo-C8 adducts of PhIP (**37**, Figure 3), 2-amino-α-carboline (**38**, Figure 3), MeIQx (**39**, Figure 3), and 4-aminobiphenyl (**20**, Figure 2) were characterized and quantified using consecutive reaction monitoring. The PhIP-DNA adducts were detected most frequently, in saliva samples from 13 of 29 ever-smokers, and in 2 of 8 samples from never-smokers. Levels of these adducts ranged from 1 to 9 per 10^8 DNA bases.

Alcohol consumption is an established risk factor for cancer of the upper aerodigestive tract, including oral cavity cancer. Acetaldehyde, the primary metabolite of alcohol, is considered to be crucial in DNA damage by alcohol and is regarded by the International Agency for Research on Cancer as "carcinogenic to humans" when associated with the consumption of alcoholic beverages.[55] The major DNA adduct of acetaldehyde is N^2-ethylidene-dGuo (**21**, Figure 2), which can be quantified by LC-MS/MS as N^2-ethyl-dGuo in DNA that has been treated with $NaBH_3CN$. Our group quantified levels of this adduct in oral cells collected by mouthwash at various time points after consumption by nonsmokers of increasing alcohol doses, administered as vodka to reach target blood alcohol concentrations of 0.03−0.07%.[56] Levels of N^2-ethylidene-dGuo in oral cell DNA increased as much as 100-fold from baseline within 4 h after each dose for all subjects and in a dose-responsive manner, reaching levels as high as 1 adduct per 10^5 nucleotides. A time dependent increase and decrease in levels of this adduct in oral cells was clearly observed after all three alcohol doses. Levels of the adduct also increased in lymphocytes and granulocytes of the subjects, but there was substantial intraindividual variability which obscured clear elucidation of alcohol's effects.[57] Acetaldehyde exposure from alcohol consumption is expected to greatly exceed that from smoking, but further studies are needed on the potential interaction of drinking and smoking on oral cell DNA adduct levels.

The tobacco-specific nitrosamines NNK (**6**, Figure 1) and NNN (**7**, Figure 1) undergo metabolic α-hydroxylation reactions catalyzed by cytochrome P450 enzymes yielding a pyridyloxobutylating intermediate which reacts with cellular DNA to produce a variety of characterized adducts with dGuo, dThd, and dCyd.[58] Treatment of this DNA with acid causes hydrolysis of several pyridyloxobutyl adducts (see **32**, Figure 2) with the release of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB). Thus, HPB-releasing DNA adducts are a measure of pyridyloxobutylation of DNA by NNK, NNN, and possibly other tobacco-specific compounds. Our group quantified HPB-releasing DNA adducts in oral cells collected by buccal brushing and by mouthwash.[59] In smokers' samples collected by mouthwash, the levels of HPB-releasing DNA adducts averaged 12.0 pmol HPB/mg DNA (4 adducts per 10^6 nucleotides), and they were detected in 20 of the 28 samples with quantifiable yield of DNA. Samples were also collected by buccal brushing, and the levels of adducts correlated with those collected by mouthwash and averaged 44.7 pmol HPB/mg DNA (15 adducts per 10^6 nucleotides) in smokers. HPB-releasing DNA adducts in nonsmoker samples were mostly undetectable.

Overall, there are no coordinated studies of DNA adducts in human oral tissue and saliva vs levels of the same adducts in human lung. Figure 2 demonstrates that there are 10 DNA adducts that have been detected in both human oral tissue or saliva and human lung, but never from the same humans. Figure 3 demonstrates that there are 7 adducts that have been detected in human saliva but have not been reported in human lung. It would most likely be impractical to carry out a study in which adducts from lung tissue and oral tissue or saliva from the same human were analyzed. Therefore, it will be necessary in future studies to depend on the assumption, supported by the studies discussed in the previous section, that genetic alterations in oral tissue reflect those in the lung because both are in the field affected by cigarette smoke. The relationship of salivary DNA adducts to adduct levels in the lung may be more complex.

■ DEVELOPING A RESEARCH APPROACH

The development of a panel of oral cell DNA adducts that might predict lung cancer susceptibility is indeed challenging. The first challenge is the selection of DNA adducts to be quantified. Considering what is known about tobacco smoke carcinogenesis, it would be prudent to include adducts derived from a variety of different types of carcinogens and relevant enhancing processes. Tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons, and volatiles such as formaldehyde,

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

acetaldehyde, 1,3-butadiene, and acrolein are representative of major classes of carcinogens and toxicants in cigarette smoke. Inflammation and oxidative damage are also likely to play a significant role in lung carcinogenesis by tobacco smoke. Thus, as an initial approach, one could consider a panel of DNA adducts consisting of HPB-releasing DNA adducts (**32**, Figure 2), benzo[*a*]pyrene-DNA adducts (**22**, Figure 2) or their hydrolysis products (such as benzo[*a*]pyrene tetraols), formaldehyde-DNA adducts, 1,3-butadiene-DNA adducts, acrolein-DNA adducts (**23** and **25**, Figure 2), 8-oxo-dGuo (**19**, Figure 2), and etheno-dGuo (**33**, Figure 3). Among these, only the HPB-releasing DNA adducts, acrolein-DNA adducts, and 8-oxo-dGuo have been identified and quantified in human oral tissues. Formaldehyde-DNA adducts have been quantified in human leukocyte DNA,[60] benzo[*a*]pyrene-DNA adducts have been occasionally quantified in human lung DNA,[46,61] and etheno-dGuo has been measured in human salivary DNA.[51] Considering that only 1−10 μg of oral cell DNA would be available from a typical collection, the quantitation of multiple adducts, some of which are typically found at levels of 1 per 10^8 nucleotides, represents a major analytical chemistry challenge. It is now realistic to consider addressing this challenge because contemporary nanoflow-LC-nanoelectrospray ionization techniques coupled with state of the art mass spectrometers can achieve detection limits in the low amol, or even high zmol range, sufficient for this type of analysis, although it still may require multiple collections per individual.

Each method must be fully validated for accuracy, precision, linearity, ruggedness, and freedom from artifacts. Another variable is the site and method of sample collection from the oral cavity. It may also be necessary to explore the contribution of oral microorganisms to DNA adduct formation. When each method has been validated and shown not to result from the possible contribution of adducted bacterial DNA, it would be tested for inclusion in the panel. The first test would be comparison of DNA adduct levels from smokers versus nonsmokers. If the DNA adduct is not elevated in smokers or if there is not a sufficient range of values in smokers, it would not likely be a valuable addition to the panel. Typically, an initial evaluation of this type requires at least 30 samples from smokers and 30 from nonsmokers, which could then be confirmed in larger studies.[59,60,62] The second test would be longitudinal or temporal stability of the DNA adduct level. This is important because the adduct levels are proposed to reflect the balance between DNA adduct formation and repair. If this is the case, it would be expected that the DNA adduct level would remain relatively constant over time, if the cigarette smoker maintains his/her habit. Studies of this type have been performed for urinary metabolites, and it would be important to carry out similar studies for the oral cell DNA adducts. For example, we showed that the intraclass correlation coefficient for urinary total NNAL was 76% in 70 smokers sampled every other month for one year indicating relative stability of these measurements in a given individual.[63] When these tests of the oral cell DNA adducts would be completed, the panel could be applied in prospective epidemiology studies to test its ability in risk prediction. Prospective epidemiology studies of lung cancer with collected oral cells include the Shanghai Cohort study, the Multi-Ethnic Cohort study, the Wayne State University and Karmanos Cancer Institute study, the University of Toronto study, the Los Angeles population-based case-control study, and the UCSF Northern California Lung Cancer study.[64] These studies also collect extensive data on factors such as diet and alcohol consumption which would be included in the final statistical model.

While the development of a panel of oral cell DNA adducts is clearly challenging, the same could have been said about the panel of urinary metabolites summarized in Table 1. In practice, these biomarkers as well as a number of additional ones are now routinely measured in large studies of tobacco smoke toxicant and carcinogen exposure. The Population Assessment of Tobacco and Health (PATH) study, jointly sponsored by the National Institutes of Health and the Food and Drug Administration, follows 46,000 people, some of whom are tobacco users, some of whom are not, for at least 3 years. The PATH study thus generates many thousands of urine samples which are being analyzed in the laboratories of the Centers for Disease Control and Prevention using mass spectrometry-based robotically driven analytical systems. Our own relatively small laboratory is able to quantify thousands of urinary biomarker samples from smokers using high throughput technology coupled to mass spectrometry and starting with small amounts of urine, typically 1−2 mL.[65,66] These activities would have been incomprehensible in the early part of this century.

Exposure parameters and oral cell DNA adducts would likely only be part of an ultimate model for susceptibility to lung cancer, and it is recognized that these could be affected by polymorphisms in carcinogen metabolizing genes such as glutathione-*S*-transferases or UDP-glucuronosyl transferases. The model would also need to include genotyping data for *CYP2A6*, the highly polymorphic major enzyme involved in nicotine metabolism, with low activity forms affecting smoking behavior, nicotine uptake, and lung cancer risk.[67] It would likely also include genotyping for variation in the α5 nicotinic cholinergic receptor subunit gene (*CHRNA5*) associated with nicotine dependence.[64] The association with quantity smoked has been reported for rs16969968 and rs1051730, which are correlated genetic variants. These variants are also associated with lung cancer and COPD,[64] and a haplotype rs588765-rs16969968 is also significantly associated with lung cancer (2−3-fold increased risk).[68] Some studies have examined the use of these and related SNPs to assess efficacy in smoking cessation.[69−71] Additional findings could be included as they evolve in genomics and epigenetics studies. For example, hypomethylation of CpG sites in smoking-related genes such as the *AHRR* gene has been shown to be associated with future lung cancer in some prospective epidemiology studies.[72]

■ SUMMARY

This perspective highlights the potential use of oral cell DNA adducts to enhance the predictive power of a proposed model to identify, at a relatively young age, those individuals highly susceptible to lung cancer. Oral cell DNA is easily obtained and is part of the physiological field affected by tobacco smoke, which includes the lung and upper aerodigestive tract, all of which are highly susceptible to tobacco induced cancer. Oral cell DNA adduct levels would complement the existing panel of urinary tobacco carcinogen and toxicant metabolites which provide excellent exposure data but only limited information on metabolic activation. Oral cell DNA adducts could potentially identify those individuals who have relatively high DNA adduct loads and consequently are more likely to harbor mutated *KRAS* and *TP53* genes, the most commonly mutated genes in smoking associated lung cancer.

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

AUTHOR INFORMATION

Corresponding Author

*Masonic Cancer Center, University of Minnesota, 2231 6th Street SE, 2-148 CCRB, Minneapolis, MN 55455, USA. Phone: 612-624-7604. Fax: 612-624-3869. E-mail: hecht002@umn.edu.

ORCID

Stephen S. Hecht: 0000-0001-7228-1356

Funding

This research is supported by grants CA-81301 and CA-138338 from the U.S. National Cancer Institute.

Notes

The author declares no competing financial interest.

Biography

Stephen S. Hecht received his B.S. in chemistry from Duke University and his Ph.D. in organic chemistry from the Massachusetts Institute of Technology, where he also did postdoctoral research in mass spectrometry. Prior to moving to the University of Minnesota in 1996, he conducted research at the American Health Foundation in Valhalla, NY for 23 years, and was Director of Research from 1987−1996. At the University of Minnesota, he served as Head of the Carcinogenesis and Chemoprevention Program of the Masonic Cancer Center from 1997−2014 and is a current member of the Medicinal Chemistry and Pharmacology graduate programs.

ACKNOWLEDGMENTS

I thank Bob Carlson and Adam Zarth for editorial assistance.

ABBREVIATIONS

NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*′-nitrosonornicotine; PheT, *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene; SPMA, *S*-phenylmercapturic acid; HPMA, 3-hydroxypropylmercapturic acid; HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; methyl-IQx, 2-amino-3,8-dimethyl-3*H*-imidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; HPB, 4-hydroxy-1-(3-pyridyl)-1-butanone; *CHRNA5*, α5 nicotinic cholinergic receptor subunit gene

REFERENCES

(1) Eriksen, M., Mackay, J., Schluger, N., Islami Gomeshtapeh, F., and Drope, J. (2015) *The Tobacco Atlas*, 5th ed., pp 32−35, American Cancer Society and World Lung Foundation, Atlanta, GA.

(2) Jamal, A., Homa, D. M., O'Connor, E., Babb, S. D., Caraballo, R. S., Singh, T., Hu, S. S., and King, B. A. (2015) Current cigarette smoking among adults - United States, 2005−2014. *MMWR Morb. Mortal. Wkly. Rep. 64*, 1233−1240.

(3) United States Department of Health and Human Services (2014) *The Health Consequences of Smoking: 50 Years of Progress. A Report of the Surgeon General*, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, Atlanta, GA.

(4) Hirsch, F. R., Scagliotti, G. V., Mulshine, J. L., Kwon, R., Curran, W. J., Jr., Wu, Y. L., and Paz-Ares, L. (2016) Lung cancer: current therapies and new targeted treatments. *Lancet*, DOI: 10.1016/S0140-6736(16)30958-8.

(5) Siegel, R. L., Miller, K. D., and Jemal, A. (2016) Cancer statistics, 2016. *Ca-Cancer J. Clin. 66*, 7−30.

(6) Stewart, B. W., and Wild, C. P. (2014) *World Cancer Report 2014*, IARC, Lyon, France.

(7) Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., and Forman, D. (2011) Global cancer statistics. *Ca-Cancer J. Clin. 61*, 69−90.

(8) International Agency for Research on Cancer (2004) Tobacco Smoke and Involuntary Smoking, in *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. *83*, pp 174−176, IARC, Lyon, France.

(9) Hayes, S. A., Haefliger, S., Harris, B., Pavlakis, N., Clarke, S. J., Molloy, M. P., and Howell, V. M. (2016) Exhaled breath condensate for lung cancer protein analysis: a review of methods and biomarkers. *J. Breath. Res. 10*, 034001.

(10) Tammemagi, C. M., Pinsky, P. F., Caporaso, N. E., Kvale, P. A., Hocking, W. G., Church, T. R., Riley, T. L., Commins, J., Oken, M. M., Berg, C. D., and Prorok, P. C. (2011) Lung cancer risk prediction: prostate, lung, colorectal and ovarian cancer screening trial models and validation. *J. Natl. Cancer Inst. 103*, 1058−1068.

(11) Etzel, C. J., and Bach, P. B. (2011) Estimating individual risk for lung cancer. *Semin. Respir. Crit Care Med. 32*, 3−9.

(12) Spitz, M. R., Etzel, C. J., Dong, Q., Amos, C. I., Wei, Q., Wu, X., and Hong, W. K. (2008) An expanded risk prediction model for lung cancer. *Cancer Prev. Res. 1*, 250−254.

(13) Cassidy, A., Myles, J. P., van Tongeren, M., Page, R. D., Liloglou, T., Duffy, S. W., and Field, J. K. (2008) The LLP risk model: an individual risk prediction model for lung cancer. *Br. J. Cancer 98*, 270−276.

(14) Bach, P. B., Kattan, M. W., Thornquist, M. D., Kris, M. G., Tate, R. C., Barnett, M. J., Hsieh, L. J., and Begg, C. B. (2003) Variations in lung cancer risk among smokers. *J. Natl. Cancer Inst. 95*, 470−478.

(15) Cronin, K. A., Gail, M. H., Zou, Z., Bach, P. B., Virtamo, J., and Albanes, D. (2006) Validation of a model of lung cancer risk prediction among smokers. *J. Natl. Cancer Inst. 98*, 637−640.

(16) Weissfeld, J. L., Lin, Y., Lin, H. M., Kurland, B. F., Wilson, D. O., Fuhrman, C. R., Pennathur, A., Romkes, M., Nukui, T., Yuan, J. M., Siegfried, J. M., and Diergaarde, B. (2015) Lung cancer risk prediction using common SNPs located in GWAS-identified susceptibility regions. *J. Thorac. Oncol. 10*, 1538−1545.

(17) Li, W., Wang, Y., Zhang, Q., Tang, L., Liu, X., Dai, Y., Xiao, L., Huang, S., Chen, L., Guo, Z., Lu, J., and Yuan, K. (2015) MicroRNA-486 as a biomarker for early diagnosis and recurrence of non-small cell lung cancer. *PLoS One 10*, e0134220.

(18) Li, K., Husing, A., Sookthai, D., Bergmann, M., Boeing, H., Becker, N., and Kaaks, R. (2015) Selecting high-risk individuals for lung cancer screening: a prospective evaluation of existing risk models and eligibility criteria in the German EPIC cohort. *Cancer Prev. Res. 8*, 777−785.

(19) Baldwin, D. R. (2015) Prediction of risk of lung cancer in populations and in pulmonary nodules: Significant progress to drive changes in paradigms. *Lung Cancer. 89*, 1−3.

(20) Marcus, M. W., Raji, O. Y., and Field, J. K. (2015) Lung cancer screening: identifying the high risk cohort. *J. Thorac. Dis. 7*, S156−S162.

(21) Hecht, S. S., Yuan, J.-M., and Hatsukami, D. K. (2010) Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. *Chem. Res. Toxicol. 23*, 1001−1008.

(22) Murphy, S. E., Park, S.-S. L., Thompson, E. F., Wilkens, L. R., Patel, Y., Stram, D. O., and Le Marchand, L. (2014) Nicotine *N*-glucurionidation relative to *N*-oxidation and *C*-oxidation and UGT2B10 genotype in five ethnic/racial groups. *Carcinogenesis 35*, 2526−2533.

(23) Park, S. L., Carmella, S. G., Ming, X., Stram, D. O., Le Marchand, L., and Hecht, S. S. (2015) Variation in levels of the lung carcinogen NNAL and its glucuronides in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. *Cancer Epidemiol., Biomarkers Prev. 24*, 561−569.

(24) Park, S. L., Carmella, S. G., Chen, M., Patel, Y., Stram, D. O., Haiman, C. A., LeMarchand, L., and Hecht, S. S. (2015) Mercpaturic acids derived from the toxicants acrolein and crotonaldehyde in the urine of cigarettes smokers from five ethnic groups with differing risks for lung cancer. *PLoS One 10*, e0124841.

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

(25) Patel, Y. M., Park, S. L., Carmella, S. G., Paiano, V., Olvera, N., Stram, D. O., Haiman, C. A., Le Marchand, L., and Hecht, S. S. (2016) Metabolites of the polycyclic aromatic hydrocarbon phenanthrene in the urine of cigarette smokers from five ethnic groups with differing risks for lung cancer. *PLoS One 11*, e0156203.
(26) Yuan, J. M., Butler, L. M., Stepanov, I., and Hecht, S. S. (2014) Urinary tobacco smoke-constituent biomarkers for assessing risk of lung cancer. *Cancer Res. 74*, 401−411.
(27) Hecht, S. S., Murphy, S. E., Stepanov, I., Nelson, H. H., and Yuan, J.-M. (2013) Tobacco smoke biomarkers and cancer risk among male smokers in the Shanghai Cohort Study. *Cancer Lett. 334*, 34−38.
(28) Proia, N. K., Paszkiewicz, G. M., Nasca, M. A., Franke, G. E., and Pauly, J. L. (2006) Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer–a review. *Cancer Epidemiol., Biomarkers Prev. 15*, 1061−1077.
(29) Garcia-Closas, M., Egan, K. M., Abruzzo, J., Newcomb, P. A., Titus-Ernstoff, L., Franklin, T., Bender, P. K., Beck, J. C., Le Marchand, L., Lum, A., Alavanja, M., Hayes, R. B., Rutter, J., Buetow, K., Brinton, L. A., and Rothman, N. (2001) Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiol. Biomarkers Prev. 10*, 687−696.
(30) Tan, D., Goerlitz, D. S., Dumitrescu, R. G., Han, D., Seillier-Moiseiwitsch, F., Spernak, S. M., Orden, R. A., Chen, J., Goldman, R., and Shields, P. G. (2008) Associations between cigarette smoking and mitochondrial DNA abnormalities in buccal cells. *Carcinogenesis 29*, 1170−1177.
(31) Borthakur, G., Butryee, C., Stacewicz-Sapuntzakis, M., and Bowen, P. E. (2008) Exfoliated buccal mucosa cells as a source of DNA to study oxidative stress. *Cancer Epidemiol., Biomarkers Prev. 17*, 212−219.
(32) Bhutani, M., Pathak, A. K., Fan, Y. H., Liu, D. D., Lee, J. J., Tang, H., Kurie, J. M., Morice, R. C., Kim, E. S., Hong, W. K., and Mao, L. (2008) Oral epithelium as a surrogate tissue for assessing smoking-induced molecular alterations in the lungs. *Cancer Prev. Res. 1*, 39−44.
(33) Kumar, S. V., Jain, R., Mokhiber, K., Venezia, A., Sheehan, A., and Spivack, S. D. (2005) Exfoliated buccal and microdissected lung cell expression of antioxidant enzymes. *Cancer Detect. Prev. 29*, 552−561.
(34) Sridhar, S., Schembri, F., Zeskind, J., Shah, V., Gustafson, A. M., Steiling, K., Liu, G., Dumas, Y. M., Zhang, X., Brody, J. S., Lenburg, M. E., and Spira, A. (2008) Smoking-induced gene expression changes in the bronchial airway are reflected in nasal and buccal epithelium. *BMC Genomics 9*, 259.
(35) Boyle, J. O., Gumus, Z. H., Kacker, A., Choksi, V. L., Bocker, J. M., Zhou, X. K., Yantiss, R. K., Hughes, D. B., Du, B., Judson, B. L., Subbaramaiah, K., and Dannenberg, A. J. (2010) Effects of cigarette smoke on the human oral mucosal transcriptome. *Cancer Prev. Res. 3*, 266−278.
(36) Roy, H. K., Subramanian, H., Damania, D., Hensing, T. A., Rom, W. N., Pass, H. I., Ray, D., Rogers, J. D., Bogojevic, A., Shah, M., Kuzniar, T., Pradhan, P., and Backman, V. (2010) Optical detection of buccal epithelial nanoarchitectural alterations in patients harboring lung cancer: implications for screening. *Cancer Res. 70*, 7748−7754.
(37) Teschendorff, A. E., Yang, Z., Wong, A., Pipinikas, C. P., Jiao, Y., Jones, A., Anjum, S., Hardy, R., Salvesen, H. B., Thirlwell, C., Janes, S. M., Kuh, D., and Widschwendter, M. (2015) Correlation of smoking-associated DNA methylation changes in buccal cells with DNA methylation changes in epithelial cancer. *JAMA Oncol. 1*, 476−485.
(38) Sidransky, D. (2008) The oral cavity as a molecular mirror of lung carcinogenesis. *Cancer Prev. Res. 1*, 12−14.
(39) Spira, A. (2010) Upper airway gene expression in smokers: the mouth as a "window to the soul" of lung carcinogenesis? *Cancer Prev. Res. 3*, 255−258.
(40) Phillips, D. H., and Venitt, S. (2012) DNA and protein adducts in human tissues resulting from exposure to tobacco smoke. *Int. J. Cancer 131*, 2733−2753.
(41) Phillips, D. H. (2002) Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis 23*, 1979−2004.
(42) Hecht, S. S. (2012) Lung carcinogenesis by tobacco smoke. *Int. J. Cancer 131*, 2724−2732.
(43) Grigoryeva, E. S., Kokova, D. A., Gratchev, A. N., Cherdyntsev, E. S., Buldakov, M. A., Kzhyshkowska, J. G., and Cherdyntseva, N. V. (2015) Smoking-related DNA adducts as potential diagnostic markers of lung cancer: new perspectives. *Exp. Oncol. 37*, 5−12.
(44) Arif, J. M., Dresler, C., Clapper, M. L., Gairola, C. G., Srinivasan, C., Lubet, R. A., and Gupta, R. C. (2006) Lung DNA adducts detected in human smokers are unrelated to typical polyaromatic carcinogens. *Chem. Res. Toxicol. 19*, 295−299.
(45) United States Department of Health and Human Services (2010) *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*, Ch. 5, pp 242−244, U.S. Department of Health and Human Services, Washington, D.C.
(46) Beland, F. A., Churchwell, M. I., Von Tungeln, L. S., Chen, S., Fu, P. P., Culp, S. J., Schoket, B., Gyorffy, E., Minarovits, J., Poirier, M. C., Bowman, E. D., Weston, A., and Doerge, D. R. (2005) High-performance liquid chromatography electrospray ionization tandem mass spectrometry for the detection and quantitation of benzo[a]-pyrene-DNA adducts. *Chem. Res. Toxicol. 18*, 1306−1315.
(47) Bessette, E. E., Goodenough, A. K., Langouet, S., Yasa, I., Kozekov, I. D., Spivack, S. D., and Turesky, R. J. (2009) Screening for DNA adducts by data-dependent constant neutral loss-triple stage mass spectrometry with a linear quadrupole ion trap mass spectrometer. *Anal. Chem. 81*, 809−819.
(48) Nath, R. G., Ocando, J. E., Guttenplan, J. B., and Chung, F. L. (1998) 1,N^2-Propanodeoxyguanosine adducts: Potential new biomarkers of smoking-induced DNA damage in human oral tissue. *Cancer Res. 58*, 581−584.
(49) Greenspan, E. J., Lee, H., Dyba, M., Pan, J., Mekambi, K., Johnson, T., Blancato, J., Mueller, S., Berry, D. L., and Chung, F. L. (2012) High-throughput, quantitative analysis of acrolein-derived DNA adducts in human oral cells by immunohistochemistry. *J. Histochem. Cytochem. 60*, 844−853.
(50) Pan, J., Awoyemi, B., Xuan, Z., Vohra, P., Wang, H. T., Dyba, M., Greenspan, E., Fu, Y., Creswell, K., Zhang, L., Berry, D., Tang, M. S., and Chung, F. L. (2012) Detection of acrolein-derived cyclic DNA adducts in human cells by monoclonal antibodies. *Chem. Res. Toxicol. 25*, 2788−2795.
(51) Chen, H. J., and Lin, W. P. (2011) Quantitative analysis of multiple exocyclic DNA adducts in human salivary DNA by stable isotope dilution nanoflow liquid chromatography-nanospray ionization tandem mass spectrometry. *Anal. Chem. 83*, 8543−8551.
(52) Chen, H. J., and Lin, C. R. (2014) Noninvasive measurement of smoking-associated N(3)-ethyladenine and N(7)-ethylguanine in human salivary DNA by stable isotope dilution nanoflow liquid chromatography-nanospray ionization tandem mass spectrometry. *Toxicol. Lett. 225*, 27−33.
(53) Chen, H. J., and Lee, C. R. (2014) Detection and simultaneous quantification of three smoking-related ethylthymidine adducts in human salivary DNA by liquid chromatography tandem mass spectrometry. *Toxicol. Lett. 224*, 101−107.
(54) Bessette, E. E., Spivack, S. D., Goodenough, A. K., Wang, T., Pinto, S., Kadlubar, F. F., and Turesky, R. J. (2010) Identification of carcinogen DNA adducts in human saliva by linear quadrupole ion trap/multistage tandem mass spectrometry. *Chem. Res. Toxicol. 23*, 1234−1244.
(55) International Agency for Research on Cancer (2012) Personal Habits and Indoor Combustions, in *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. *100E*, pp 373−499, IARC, Lyon, France.
(56) Balbo, S., Meng, L., Bliss, R. L., Jensen, J. A., Hatsukami, D. K., and Hecht, S. S. (2012) Kinetics of DNA adduct formation in the oral cavity after drinking alcohol. *Cancer Epidemiol., Biomarkers Prev. 21*, 601−608.
(57) Balbo, S., Meng, L., Bliss, R. L., Jensen, J. A., Hatsukami, D. K., and Hecht, S. S. (2012) Time course of DNA adduct formation in

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

peripheral blood granulocytes and lymphocytes after drinking alcohol. *Mutagenesis 27*, 485−490.
(58) Hecht, S. S. (2008) Progress and challenges in selected areas of tobacco carcinogenesis. *Chem. Res. Toxicol. 21*, 160−171.
(59) Stepanov, I., Muzic, J., Le, C. T., Sebero, E., Villalta, P., Ma, B., Jensen, J., Hatsukami, D. K., and Hecht, S. S. (2013) Analysis of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB)-releasing DNA adducts in human exfoliated oral mucosa cells by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Chem. Res. Toxicol. 26*, 37−45.
(60) Wang, M., Cheng, G., Balbo, S., Carmella, S. G., Villalta, P. W., and Hecht, S. S. (2009) Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and non-smokers. *Cancer Res. 69*, 7170−7174.
(61) Alexandrov, K., Cascorbi, I., Rojas, M., Bouvier, G., Kriek, E., and Bartsch, H. (2002) CYP1A1 and GSTM1 genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis 23*, 1969−1977.
(62) Zhang, S., Balbo, S., Wang, M., and Hecht, S. S. (2011) Analysis of acrolein-derived 1,N^2-propanodeoxyguanosine adducts in human leukocyte DNA from smokers and nonsmokers. *Chem. Res. Toxicol. 24*, 119−124.
(63) Church, T. R., Anderson, K. E., Le, C., Zhang, Y., Kampa, D. M., Benoit, A. R., Yoder, A. R., Carmella, S. G., and Hecht, S. S. (2010) Temporal stability of urinary and plasma biomarkers of tobacco smoke exposure among cigarette smokers. *Biomarkers 15*, 345−352.
(64) Chen, L. S., Hung, R. J., Baker, T., Horton, A., Culverhouse, R., Saccone, N., Cheng, I., Deng, B., Han, Y., Hansen, H. M., Horsman, J., Kim, C., Lutz, S., Rosenberger, A., Aben, K. K., Andrew, A. S., Breslau, N., Chang, S. C., Dieffenbach, A. K., Dienemann, H., Frederiksen, B., Han, J., Hatsukami, D. K., Johnson, E. O., Pande, M., Wrensch, M. R., McLaughlin, J., Skaug, V., van der Heijden, H. F., Wampfler, J., Wenzlaff, A., Woll, P., Zienolddiny, S., Bickeboller, H., Brenner, H., Duell, E. J., Haugen, A., Heinrich, J., Hokanson, J. E., Hunter, D. J., Kiemeney, L. A., Lazarus, P., Le Marchand, L., Liu, G., Mayordomo, J., Risch, A., Schwartz, A. G., Teare, D., Wu, X., Wiencke, J. K., Yang, P., Zhang, Z. F., Spitz, M. R., Kraft, P., Amos, C. I., and Bierut, L. J. (2015) CHRNA5 risk variant predicts delayed smoking cessation and earlier lung cancer diagnosis–a meta-analysis. *J. Natl. Cancer Inst. 107*, pii: djv100.
(65) Carmella, S. G., Ming, X., Olvera, N., Brookmeyer, C., Yoder, A., and Hecht, S. S. (2013) High throughput liquid and gas chromatography-tandem mass spectrometry assays for tobacco-specific nitrosamine and polycyclic aromatic hydrocarbon metabolites associated with lung cancer in smokers. *Chem. Res. Toxicol. 26*, 1209−1217.
(66) Carmella, S. G., Chen, M., Zarth, A., and Hecht, S. S. (2013) High throughput liquid chromatography-tandem mass spectrometry assay for mercapturic acids of acrolein and crotonaldehyde in cigarette smokers' urine. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 935*, 36−40.
(67) Yuan, J.-Y., Nelson, H. H., Butler, L. M., Carmella, S. G., Wang, R., Kuriger-Laber, J., Adams-Haduch, J., Hecht, S. S., Gao, Y.-T., and Murphy, S. E. (2016) Genetic determinants of cytochrome P450 2A6 and biomarkers of tobacco smoke exposure in releation to risk of lung cancer development in the Shanghai Cohort Study. *Int. J. Cancer 138*, 2161−2171.
(68) Ji, X., Gui, J., Han, Y., Brennan, P., Li, Y., McKay, J., Caporaso, N., Bertazzi, P. A., Landi, M. T., and Amos, C. I. (2015) The role of haplotype in 15q25.1 locus in lung cancer risk: Results of scanning chromosome 15. *Carcinogenesis 36*, 1275−1273.
(69) Chen, L. S., Baker, T. B., Jorenby, D., Piper, M., Saccone, N., Johnson, E., Breslau, N., Hatsukami, D., Carney, R. M., and Bierut, L. J. (2015) Genetic variation (CHRNA5), medication (combination nicotine replacement therapy vs. varenicline), and smoking cessation. *Drug Alcohol Depend. 154*, 278−282.
(70) Bierut, L. J., Johnson, E. O., and Saccone, N. L. (2014) A glimpse into the future - Personalized medicine for smoking cessation. *Neuropharmacology 76*, 592−599.
(71) Zhu, A. Z., Zhou, Q., Cox, L. S., David, S. P., Ahluwalia, J. S., Benowitz, N. L., and Tyndale, R. F. (2014) Association of CHRNA5-A3-B4 SNP rs2036527 with smoking cessation therapy response in African-American smokers. *Clin. Pharmacol. Ther. 96*, 256−265.
(72) Fasanelli, F., Baglietto, L., Ponzi, E., Guida, F., Campanella, G., Johansson, M., Grankvist, K., Johansson, M., Assumma, M. B., Naccarati, A., Chadeau-Hyam, M., Ala, U., Faltus, C., Kaaks, R., Risch, A., De Stavola, B., Hodge, A., Giles, G. G., Southey, M. C., Relton, C. L., Haycock, P. C., Lund, E., Polidoro, S., Sandanger, T. M., Severi, G., and Vineis, P. (2015) Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. *Nat. Commun. 6*, 10192.
(73) Hukkanen, J., Jacob, P., and Benowitz, N. L. (2005) Metabolism and disposition kinetics of nicotine. *Pharmacol. Rev. 57*, 79−115.
(74) Yuan, J. M., Koh, W. P., Murphy, S. E., Fan, Y., Wang, R., Carmella, S. G., Han, S., Wickham, K., Gao, Y. T., Yu, M. C., and Hecht, S. S. (2009) Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. *Cancer Res. 69*, 2990−2995.
(75) Yuan, J.-M., Gao, Y.-T., Murphy, S. E., Carmella, S. G., Wang, R., Zhong, Y., Moy, K. A., Davis, A. B., Tao, L., Chen, M., Han, S., Nelson, H. H., Yu, M. C., and Hecht, S. S. (2011) Urinary levels of cigarette smoke constituent metabolites are prospectively associated with lung cancer development in smokers. *Cancer Res. 71*, 6749−6757.
(76) Church, T. R., Anderson, K. E., Caporaso, N. E., Geisser, M. S., Le, C., Zhang, Y., Benoit, A. R., Carmella, S. G., and Hecht, S. S. (2009) A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. *Cancer Epidemiol., Biomarkers Prev. 18*, 260−266.
(77) Carmella, S. G., Chen, M., Yagi, H., Jerina, D. M., and Hecht, S. S. (2004) Analysis of phenanthrols in human urine by gas chromatography-mass spectrometry: potential use in carcinogen metabolite phenotyping. *Cancer Epidemiol. Biomarkers Prev. 13*, 2167−2174.
(78) Carmella, S. G., Chen, M., Han, S., Briggs, A., Jensen, J., Hatsukami, D. K., and Hecht, S. S. (2009) Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. *Chem. Res. Toxicol. 22*, 734−741.
(79) Haiman, C. A., Patel, Y. M., Stram, D. O., Carmella, S. G., Chen, M., Wilkens, L. R., Le Marchand, L., and Hecht, S. S. (2016) Benzene uptake and glutathione *S*-transferase T1 status as determinants of *S*-phenylmercapturic acid in cigarette smokers in the multiethnic cohort. *PLoS One 11*, e0150641.
(80) Park, S. L., Kotapati, S., Wilkens, L. R., Tiirikainen, M., Murphy, S. E., Tretyakova, N., and Le Marchand, L. (2014) 1,3-Butadiene exposure and metabolism among Japanese American, Native Hawaiian, and white smokers. *Cancer Epidemiol., Biomarkers Prev. 23*, 2240−2249.
(81) Milne, G. L., Yin, H., Hardy, K. D., Davies, S. S., and Roberts, L. J. (2011) Isoprostane generation and function. *Chem. Rev. 111*, 5973−5996.
(82) Wang, D., and DuBois, R. N. (2013) Urinary PGE-M: a promising cancer biomarker. *Cancer Prev. Res. 6*, 507−510.

DOI: 10.1021/acs.chemrestox.6b00372
Chem. Res. Toxicol. 2017, 30, 367−375

European Journal of Cancer Prevention 1997, **6**, 226–268

REVIEW

N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids

A R Tricker

(Received 23 December 1996; accepted 19 January 1997)

Based on recent analytical data, total human exogenous exposure to *N*-nitrosamines is estimated to be 1.10 μmol/day; the major exposure sources are the diet (0.79 μmol/day, 80–120 μg/day; 72%), occupational exposure (0.15–0.30 μmol/day; 25%), cigarette smoking (0.02 μmol/day, 3.4 μg/day; 2%), and miscellaneous minor sources, including pharmaceutical products, cosmetics, indoor and outdoor air (0.001 μmol/day, 0.1 μg/day; 1%). Excretion of apparent total *N*-nitroso compounds (ATNC) in healthy adults is estimated to be 1.30 ± 1.05 μmol/day in urine and between 1.56 ± 1.56 and 3.17 ± 2.58 μmol/day in faeces. The excretion of volatile *N*-nitrosamines (*N*-nitrosodimethylamine), and *N*-nitrosamino acids and their derivatives (*N*-nitrososarcosine, *N*-nitrosoproline, *N*-nitrosothiazolidine-4-carboxylic acid and *N*-nitroso-2-methylthiazolidine-4-carboxylic acid) accounts for approximately 0.03% and 16.0% of urinary ATNC, respectively. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol and its *O*-glucuronide conjugate, two metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone present in urine of smokers, account for 0.2% of the urinary ATNC response; < 1.5% of the excretion of currently identified *N*-nitroso compounds in urine. The remaining *N*-nitroso compounds excreted in urine and those present in faeces are still unidentified. A crude mass balance between exogenous exposure and excretion in urine and faeces indicates that 45–75% of the total human exposure to *N*-nitroso compounds results through *in vivo* formation.

Key words: Biomonitoring, endogenous formation, exposure assessment, *N*-nitroso compounds.

Introduction

Prior to 1956, interest in *N*-nitroso compounds was limited to industrial applications such as the use of *N*-nitrosodimethylamine (NDMA) as a solvent in the vulcanization of rubber, and to the chemical laboratory. This situation changed rapidly after investigations of the hepatotoxicity suffered by workers in the rubber industry identified NDMA as inducing primary hepatic tumours in the rat (Magee and Barnes, 1956). Soon afterwards it became evident that *N*-nitroso compounds also occurred naturally in the environment (Ender *et al*, 1964), and a large potential existed for their endogenous formation (Sander *et al*, 1968). As a consequence, concerns were raised over the possible health risks posed by these compounds.

In 1978, the American National Academy of Sciences concluded that the endogenous formation of *N*-nitroso compounds could be the largest source of exposure for the general population (National Academy of Sciences, 1978). Three years later in 1981, the major exogenous sources of human exposure to preformed *N*-nitroso compounds were

FTR, 2003 Neuchâtel, Switzerland. Fax: (+41) 32 8887 776.

assessed to be the diet (0.3–20 μg/day), cigarette smoking (17 μg/day), cosmetics (0.4 μg/day) and occupational exposure (10–180 μg/day) (National Academy of Sciences, 1981). Since these assessments, several, but not all *N*-nitrosamines, have been shown to induce a wide range of tumours in experimental animals (Preussmann and Steward, 1984; Lijinsky, 1992). Based on limited epidemiological evidence, the potential role of *N*-nitrosamines in the aetiology of human cancers has been speculated (Preston-Martin and Correa, 1989). Several reviews have reported the mechanisms by which *N*-nitroso compounds exert their biological effects (Archer, 1989; Shuker and Bartsch, 1994) and their relevance to human cancer (Magee, 1989; Bartsch, 1991; Hoffmann *et al*, 1994; Reed, 1996).

Since 1981, the occurrence of preformed *N*-nitroso compounds in the human environment has been studied extensively (Tricker *et al*, 1989a). Based on current literature data, the 1981 exposure assessment by the National Academy of Sciences (1981) has been re-evaluated, and the major sources of human exposure calculated. Mechanisms of endogenous nitrosation, methods for its quantification, and the concentrations of *N*-nitrosamines in human body fluids are presented. Special emphasis is given to the role of dietary habits, tobacco smoking, occupational and environmental exposure to preformed *N*-nitroso compounds, and the influence of certain disease states on the concentrations of *N*-nitroso compounds in human body fluids. Finally, the excretion of apparent total *N*-nitroso compounds (ATNC) in urine and faeces is compared with the current assessment of total human exposure in order to estimate the extent of endogenous nitrosation in man.

Formation of *N*-nitroso compounds

N-nitroso compounds are characterized by a nitroso group (–N=O) bonded to a nitrogen atom. The classical method for producing an *N*-nitrosamine is the reaction of a secondary amine with nitrous acid which is formed by the reaction of nitrite under acidic conditions (Ridd, 1961; Mirvish, 1975; Williams, 1983). Nitrite and nitrous acid *per se* are not nitrosating agents but are intermediates in the formation of the nitrosating species dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4) and the nitrous acidium ion (H_2O^+NO). The acidity of the aqueous medium determines the relative proportions of the nitrosating species (Ridd, 1961).

Nitrosation of the simplest aliphatic amine, methylamine, results in a complex mixture of products which include NDMA; more complex primary aliphatic amines yield elimination, substitution and rearrangement products (Mende *et al*, 1989).

Protonated secondary amines are unreactive towards N_2O_3 and nitrosation occurs typically at an optimum of pH 3.0–3.5. Introduction of ionizable groups near to the nitrosatable amino moiety, as in the case of α-amino acids, reduces the basicity of the amine and the pH maxima for nitrosation (Mirvish *et al*, 1973). Depending on the amino acid residues present, nitrosation of dipeptides can produce *N*-nitrosodipeptides (Tricker *et al*, 1984) or low yields of *N*-nitrosoiminodialkanoic acids (Pollock, 1985). All peptides undergo nitrosation at the terminal primary amino group to yield a diazopeptide, and at the peptide N-atoms to produce *N*-nitrosopeptides (Challis, 1989). Under acidic conditions diazopeptides are usually formed more readily than *N*-nitrosopeptides, but quickly decompose. Under neutral conditions, diazopeptides, once formed, are relatively stable. *N*-Nitrosopeptides are stable under both acid and neutral conditions. Peptides containing proline, cysteine, tyrosine, tryptophan, arginine, lysine, glutamine or asparagine residues undergo additional reactions with nitrosating agents: proline and tryptophan form specific *N*-nitroso derivatives, cysteine is oxidized via an *S*-nitroso intermediate to yield a disulphide (cystine) bridge, tyrosine forms a *C*-nitroso derivative, and arginine, lysine, glutamine and asparagine are subject to deamination reactions.

Simple symmetrical trialkylamines react slowly with nitrous acid; for more complex asymmetrical amines with substituents other than simple alkyl groups, no clear systematic approach can be taken to predict which alkyl substituent will be replaced by nitrosative dealkylation. As an example, nicotine reacts very slowly *in vitro* with nitrous acid in aqueous solution to produce 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanal (NNA), *N*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Hecht *et al*, 1978), while alkaloids containing *N,N*-dialkyl side-chains and *N,N*-dialkylaromatic compounds are far more readily nitrosated (Loeppky *et al*, 1994).

Since acid catalysed nitrosation is inappreciable above about pH 5.0, *N*-nitrosamines in foodstuffs, consumer products and environmental media are most likely to be formed either by exposure to gaseous oxides of nitrogen (NOx) or via an HCHO-catalysed reaction with nitrite (Keefer and Roller, 1973). Catalysis of nitrosation may be accelerated by thiocyanate and other anions (Fine and Tannenbaum, 1973). Conversely, virtually any

compound which reacts with nitrite can inhibit the formation of *N*-nitrosamines (Bartsch *et al*, 1988). Ascorbic acid and ascorbate ions inhibit nitrosation by competing for the reaction with N_2O_3, $H_2O{\cdot}NO$ and NOx, in which ascorbic acid is oxidized irreversibly to form dehydroascorbic acid, and the nitrosating species is reduced to nitric oxide (NO) (Licht *et al*, 1988a). Tocopherols react with nitrosating species in a manner analogous to ascorbic acid; the tocopherol is oxidized to the quinone while the nitrosating species is reduced to NO (Newmark and Mergens, 1981). Inhibition of proline, hydroxyproline and sarcosine nitrosation is particularly effective at pH 1.0–4.0, although this may not apply to other amines (Chang *et al*, 1979). The formation of *N*-nitrosamines in consumer products, and current regulations to reduce their presence, has been reviewed (Challis, 1996).

Sources of exposure to *N*-nitroso compounds

Diet

Quantitatively the most abundant *N*-nitroso compounds present in the diet are non-volatile *N*-nitrosated amino acids and amino acid derivatives (NVNA). The major NVNA present in the diet are *N*-nitrososarcosine (NSAR), *N*-nitrosoproline (NPRO), *N*-nitroso-4-hydroxyproline (NHPRO), *N*-nitrosothiazolidine-4-carboxylic acid (NTCA), *N*-nitroso-2-methylthiazolidine-4-carboxylic acid (NMTCA) and *N*-nitroso-2-hydroxymethylthiazolidine-4-carboxylic acid (NHMTCA). Mechanisms for the formation and the concentrations of individual NVNA in different foodstuffs have been reviewed; these compounds occur mainly in smoked and nitrate-cured meats, dried and smoked fish, seafoods and smoked cheese at concentrations ranging between 10 and several thousand μg/kg for individual NVNA (Tricker and Kubacki, 1992). Traditionally smoked foods such as Icelandic smoked mutton contain extremely high levels of NTCA (up to 6.76 mg/kg) in addition to other *N*-nitrosamines (Helgason *et al*, 1984; Sen *et al*, 1990). Cured and smoked meats may contain NVNA formed by condensation reactions of the amino acids serine and threonine with simple aldehydes or sugars, followed by nitrosation (Tricker and Kubacki, 1992). Similar condensation reactions involving tryptophan may explain the recent report of 13–346 μg/kg 2-nitroso-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in cured and smoked meats (Sen *et al*, 1995). Although the total daily exposure to NVNA has not been determined, estimates suggest an exogenous exposure of 10–100 μg/day (Hotchkiss, 1989; Tricker and Preussmann, 1991a).

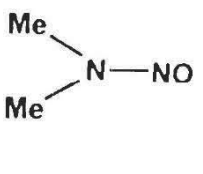
The major volatile *N*-nitrosamines (VNA) in the diet are NDMA, *N*-nitrosopyrrolidine (NPYR), *N*-nitrosopiperidine (NPIP) and *N*-nitrosothiazolidine (NTHz) (Tricker and Preussmann, 1991a). These compounds may be formed by the nitrosation of the corresponding precursor amines or thermal decarboxylation of NVNA during heat treatment of foods (Hotchkiss, 1989). Extensive dietary surveys performed in several different countries indicate that beer, cured meat and fish products contribute significantly to the total dietary exposure of 0.3–1.0 μg/day VNA (Hotchkiss, 1989; Tricker and Preussmann, 1991a). Repeated investigations of beer (Tricker and Preussmann, 1991b; Sen *et al*, 1996) and total diet surveys (Tricker *et al*, 1991a) indicate a general decreasing trend in dietary exposure to VNA.

A limited range of foods contains additional *N*-nitroso compounds, albeit at low concentrations (Tricker and Preussmann, 1991a). One source for these compounds is the migration of preformed *N*-nitrosamines and nitrosatable precursor amines from direct-contact food packaging materials (Sen, 1988); in particular, elastic rubber nettings used for meat products (Pensabene and Fiddler, 1994). Similarly, treating apples with diphenylamine may result in contamination with trace levels of *N*-nitrosodiphenylamine (Lillard and Hotchkiss, 1994). Inadvertent contamination by these sources seldom exceeds 10 μg/kg for individual *N*-nitroso compounds and makes only a negligible contribution to the total daily intake of *N*-nitrosamines. Of the 25 currently identified *N*-nitroso compounds in the diet, the major preformed *N*-nitrosamines are shown in Figure 1.

Tobacco and tobacco smoke

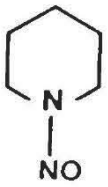
Tobacco-specific *N*-nitrosamines (TSNA) are formed during the curing and processing of tobacco by nitrosation of *Nicotiana* alkaloids (Figure 2). NNA has not been identified in tobacco or tobacco smoke, probably due to the reactivity of the aldehyde group, which could be either oxidized to yield 4-(methylnitrosamino)-4-(3-pyridyl) butyric acid (iso-NNAC) or reduced to yield 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (iso-NNAL) (Hoffmann *et al*, 1994). Reduction of the keto group in NNK yields 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). NNN is primarily formed by

Volatile *N*-nitrosamines

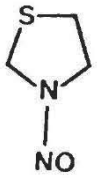


NDMA

NPYR

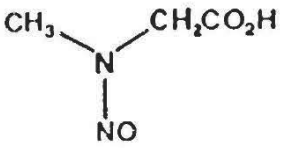


NPIP

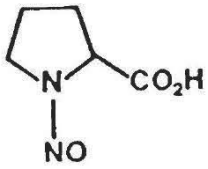


NTHz

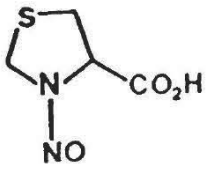
Non-volatile *N*-nitrosamino acids and derivatives



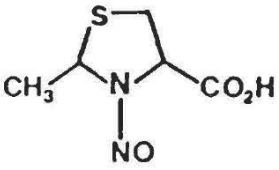
NSAR



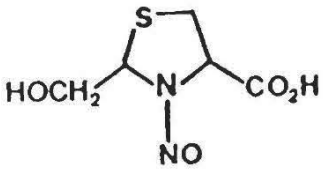
NPRO



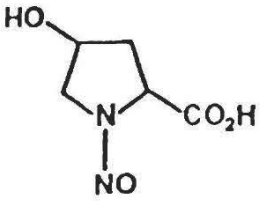
NTCA



NMTCA



NHMTCA



NHPRO

Figure 1. Major preformed *N*-nitroso compounds in the diet. Abbreviations used for the *N*-nitrosoamines are: NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NPIP, *N*-nitrosopiperidine; NTHz, *N*-nitrosothiazolidine; NSAR, *N*-nitrososarcosine; NPRO, *N*-nitrosoproline; NTCA, *N*-nitrosothiazolidine-4-carboxylic acid; NMTCA, *N*-nitroso-2-methylthiazolidine-4-carboxylic acid; NHMTCA, *N*-nitroso-2-hydroxymethylthiazolidine-4-carboxylic acid; NHPRO, *N*-nitrosohydroxyproline.

nitrosation of the tobacco alkaloid nornicotine (Mirvish *et al*, 1977), and to a lesser extent, by nitrosation of nicotine-*N*-oxide (Klimisch and Stadler, 1976). Microbial degradation of nicotine-*N*-oxide to pseudooxynicotine (Maeda *et al*, 1978), which is rapidly and irreversibly nitrosated to NNK (Caldwell *et al*, 1993), may be the major route of NNK formation in tobacco. Anabasine and anatabine are nitrosated to yield *N*-nitrosoanabasine (NAB) and *N*-nitrosoanatabine (NAT), respectively.

Commercial filter cigarettes have mean mainstream smoke deliveries of 75 ± 41 (range 19–135) ng NNN, 52 ± 29 (range 21–110) ng NNK, 73 ± 47 (range 18–155) ng NAB/NAT, 7.8 ± 2.9 (range

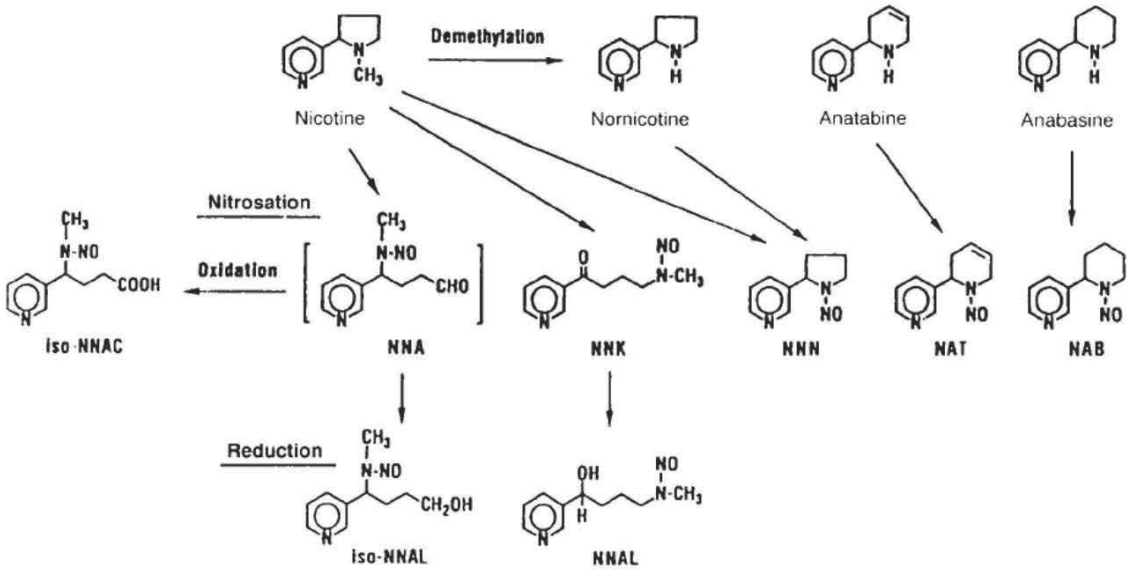


Figure 2. Formation of tobacco-specific *N*-nitrosoamines. Abbreviations used for the *N*-nitrosoamines are: NNA, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanal; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; NAT, *N*-nitrosoanatabine; NAB, *N*-nitrosoanabasine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; iso-NNAL, 4-(methylnitrosamine)-4-(3-pyridyl)-1-butanol; iso-NNAC, 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid.

5.8–15.2) ng NDMA, 2.4 ± 1.4 (range < 1.0–3.9) ng *N*-nitrosoethylmethylamine (NEMA) and 9.0 ± 3.0 (range 3.9–15.2) ng NPYR per cigarette (Tricker *et al*, 1991b). The presence of < 11 ng/cigarette iso-NNAL (Tricker *et al*, 1991b) and < 5 ng/cigarette iso-NNAC (Djordjevic *et al*, 1991; Tricker *et al*, 1993) in mainstream cigarette smoke have been less seldomly reported. Cigarette smoke does not contain NPRO (Brunnemann *et al*, 1983), and no evidence exists to show that other NVNA are present in tobacco smoke. TSNA are primarily particle-bound, while VNA occur in the gas phase (Adams *et al*, 1987). Assuming 70% retention of particle-bound TSNA in the lung during oral inhalation (Hinds *et al*, 1983), a smoker of 20 filter cigarettes per day would have a total exposure of about 3.0 μg/day TSNA (1.05 ± 0.57 μg NNN, 0.73 ± 0.41 μg NNK and 1.02 ± 0.66 μg NAB/NAT) and about 0.4 μg/day VNA.

Since TSNA and VNA are also transferred to sidestream cigarette smoke (Adams *et al*, 1987), and presumably exhaled by smokers, trace levels occur in environmental tobacco smoke (ETS) present in indoor air (Brunnenmann *et al*, 1992; Klus *et al*, 1992; Tricker *et al*, 1994). Extensive smoking under poor ventilation conditions results in mean ETS concentrations of 2.8 ± 1.6 (range ND–6.0) ng/m^3 NNN, 4.9 ± 9.6 (range ND–13.5) ng/m^3 NNK, 19.8 ± 11.4 (range 7.9–45.0) ng/m^3 NDMA and 10.0 ± 6.6 (range 3.5–27.0) ng/m^3 NPYR (Klus *et al*, 1992). NDMA has also been reported in indoor air of discotheques, bars and trains (Brunnemann and Hoffmann, 1978). Smoking within the home increases the mean background NDMA concentration from 9.5 ± 0.8 (range 8.6–10.4) to 12.5 ± 6.0 (range 3.3–25.7) ng/m^3 (Tricker *et al*, 1994). The occurrence of NDMA in homes occupied by non-smokers shows that ETS is not the only source of NDMA in indoor air. Both NDMA and NPYR are volatilized in fumes during cooking (Sen *et al*, 1976), and other combustion sources may contribute to NDMA found in indoor air (Brunnemann and Hoffmann, 1978). Assuming an adult has a daily respiratory volume of 20 m^3 and that 10–15% deposition of particle-bound TSNA occurs in the lung during nasal inhalation (Hiller *et al*, 1982), non-smoker exposure to TSNA present in ETS in a poorly ventilated office (Klus *et al*, 1992) can be estimated to be 0.4 ± 0.2 (range ND–0.8) ng/h NNN and 0.6 ± 0.4 (range ND–1.7) ng/h NNK. Therefore, over an 8 h working day exposure is unlikely to exceed more than 20 ng TSNA.

Smokeless tobacco contains seven TSNA (Hoffmann *et al*, 1994), at least 10 NVNA including

NSAR, NPRO, *N*-nitrosoazetadine-4-carboxylic acid (NAzCA) and 3-(*N*-nitrosomethylamino)propionic acid (NMPA) (Djordjevic *et al*, 1989; Tricker and Preussmann, 1991c), and various VNA (Tricker and Preussmann, 1991c). The use of smokeless tobacco is difficult to quantify, and exposure estimates range between 27.5 μg/day TSNA for users of nasal snuff tobacco (Tricker and Preussmann, 1991c) to 11.5 mg/day NNK for toombak snuff-dippers in Sudan (Idris *et al*, 1991). The presence of TSNA in the saliva of toombak users during chewing ranges from 0.7 to 30.6 μg/ml (Idris *et al*, 1992), 10- to 100-fold higher than the levels found in saliva of users of other types of smokeless tobacco (Hoffmann and Adams, 1981; Sipahimalani *et al*, 1984; Nair *et al*, 1985; Bhide *et al*, 1986; Brunnemann *et al*, 1987; Österdahl and Slorach, 1988).

Cosmetic products

During the 1970s, cosmetic products frequently contained simple VNA such as NDMA and *N*-nitrosomorpholine (NMOR) (Spiegelhalder and Preussmann, 1984) and long-chain *N*-nitroso-*N*-methylalkylamines such as *N*-nitroso-*N*-methyldodecylamine and *N*-nitroso-*N*-methyltetradecylamine (Hecht *et al*, 1982). By the beginning of the 1990s most European cosmetics were free from these contaminants (Kamp and Eisenbrand, 1991) while approximately 15% of personal care products still contained 12–235 μg/kg *N*-nitrosodiethanolamine (NDELA) and/or 40–215 μg/kg *N*-nitrosobis-(2-hydroxypropyl)amine (NDHPA) (Eisenbrand *et al*, 1991). American cosmetic products still contained 210–2,960 μg/kg NDELA, and sunscreens were reported to contain 780–5,050 μg/kg 2-ethylhexyl-4-(*N*-methylnitrosamino)benzoate (NPABAO) (Havery and Chow, 1994). Rapid photodegradation of NPABAO in sunscreen products by ultraviolet light (Havery and Chow, 1994) probably limits the extent of human exposure (Kenney *et al*, 1995); however, the photolytic decomposition products have not been identified.

With the exception of some American products, negligible exposure to *N*-nitrosodialkanolamines such as NDELA and NDHPA is predicted to occur through normal use of personal care cosmetics (European Chemical Industry Ecology and Toxicology Center, 1990). Most contemporary European personal care products with the exception of some shampoos, night creams and lipsticks containing < 50 μg/kg *N*-nitrosamines (Challis *et al*, 1995a). Changes in process chemistry and inclusion of antioxidants and iminium ion traps in cosmetic formulations are expected to result in further reductions in *N*-nitrosamine formation and contamination in the near future (Challis *et al*, 1995b).

Pharmaceutical products

In the 1970s several prescription and over-the-counter pharmaceutical products were reported to contain various VNA, in particular NDMA (Krull *et al*, 1979). More recent studies indicate considerable reductions in contamination by VNA (Dawson and Lawrence, 1987a), so that pharmaceutical products can, in general, be disregarded as a source of exogenous *N*-nitrosamine exposure. However, *N*-mononitrosopiperazine (MNPz) contamination of products containing piperazine (Dawson and Lawrence, 1987b) may still be a current problem (see following section on pharmaceutical products). Nicotine-containing chewing gum used as a smoking cessation aid has been reported to contain ~ 380 ng TSNA/mg gum (Österdahl, 1990). Other nicotine-containing pharmaceutical products available prior to 1992 also contained trace levels of TSNA (about 20–40 ng/g nicotine), but most contemporary products now appear to be free of contamination (Tricker, unpublished results).

Ambient air

Concentrations of 10–40 ng/m^3 NDMA, NDEA and NMOR have been measured in ambient air in a heavily polluted industrial area of Austria (Spiegelhalder and Preussmann, 1987). Similar levels have been reported in Germany with the highest outdoor air concentrations (10–90 ng/m^3) occurring in the vicinity of chemical plants (Akkan *et al*, 1991). Higher levels of 9–220 ng/m^3 NDMA and 11–220 ng/m^3 NDEA have been reported in ambient air of industrialized areas in the former USSR (Ianysheva *et al*, 1991). More recent data report 50–600 ng/m^3 VNA in Moscow city air with the highest concentrations found near to industrial areas (Khesina *et al*, 1996).

Occupation

During the 1970s, widespread ambient exposure to various *N*-nitrosamines also occurred in the leather industry (< 0.05–47 μg/m^3 NDMA and < 0.05–2 μg/m^3 NMOR); the rubber industry (0.02–5.5 μg/m^3 NDMA, 0.13–1.4 μg/m^3 *N*-nitrosodiethylamine (NDEA), 0.01–3.9 μg/m^3 NPYR, 0.02–250 μg/m^3 NMOR and 0.01–1,230 μg/m^3 *N*-nitrosodiphenylamine (NDPhA)); the sufactant industry (0.03–0.8 μg/m^3 NDMA); and metal foundries (0.02–1.4 μg/m^3 NDEA) (Rounbehler and

Fajen, 1982). In several cases, worker exposure to individual VNA in the rubber industry probably exceeded 50 μg/day (Spiegelhalder, 1984). Synthetic cutting fluids based on aqueous solutions of secondary and tertiary alkanolamines with added nitrite salts as corrosion inhibitors used in commercial metal-working operations contained 200–30,000 mg/kg (0.02–3% by weight) NDELA (Fan *et al*, 1977) and up to 30,000 mg/kg *N*-nitroso-5-methyl-1,3-oxazolidine (Stephany *et al*, 1978).

Reformulation of synthetic cutting fluids and discontinued use of nitrite salts as corrosion inhibitors have generally reduced contamination during storage to < 50 mg/kg NDELA (Keefer *et al*, 1990). In a recent German survey of metal workshops, concentrated cutting fluids contained a mean of 20.6 ± 5.0 (range 2–135) mg/l NDELA, and their use resulted in mean ambient air concentrations of 214 ± 391 (range < 50–1,000) ng/m^3 NDELA (Fuchs *et al*, 1995). In another recent Canadian survey of 14 metal-working factories, diluted cutting fluids contained 0.93 ± 1.96 (range 0.01–7.53) mg/l NDELA and their use resulted in airborne levels of 40.8 ± 60.2 (range ND–193) ng/m^3 NDELA (Fadlallah *et al*, 1996). The same metal-working factories also contained ambient levels of 34.4 ± 29.1 (range ND–102) ng/m^3 NDMA; 31–167 ng/m^3 NDBA was detected in four factories and 25 ng/m^3 NDEA in one factory. No correlations were observed between the NDELA concentration in cutting fluids and the levels of *N*-nitrosamines in the corresponding factory air. Contamination of diluted cutting fluids may be caused by nitrite formed by bacterial reduction of nitrate in water used for dilution and/or absorption of atmospheric NOx. The use of bismorpholinomethane as a preserving agent may be the source of 0.02–5.0 mg/l NMOR recently found in some used diluted cutting fluids (Eisenbrand *et al*, 1996). Similar to the situation with cosmetics containing alkanolamines, inclusion of antioxidants and iminium ion traps may have the potential to reduce *N*-nitrosamine formation both during storage and use in metal-working operations.

Recent area air monitoring in a German factory manufacturing rubber seals and components for automobiles determined time-weighted average concentrations of 30–120 ng/m^3 NDMA and 29–78 ng/m^3 *N*-nitrosodibenzylamine (NDBzA) (Dietrich *et al*, 1996). Concurrent to this, a survey performed by the National Institute of Occupational Safety and Health (NIOSH) measured time-weighted average concentrations of 19.2 ± 28.6 (range 2.3–88.5) μg/m^3 NDMA, 0.25 ± 0.4 (range ND–1.03) μg/m^3 NDEA, 3.89 ± 2.77 (range 1.4–10.2) μg/m^3 NPIP, 0.11 ± 0.05 (range 0.05–0.16) μg/m^3 NPYR and 0.26 ± 0.20 (range 0.06–0.55) μg/m^3 NMOR in a US factory producing rubber vehicle seals (Rey and Fajen, 1996). Total VNA concentrations averaged 23.72 ± 31.38 (range 4.50–99.5) μg/m^3. Personal breathing zone air samples collected for 28 workers showed exposure to 3.16 ± 2.47 (range 0.47–11.4) μg/m^3 NDMA, 0.17 ± 0.19 (range 0.01–0.81) μg/m^3 NDEA, 1.67 ± 0.91 (range 0.20–4.39) μg/m^3 NMOR. The average exposure to total VNA in breathing zone air samples was 5.25 ± 3.41 (range 0.76–16.3) μg/m^3. VNA concentrations in area and personal breathing zone samples were related to different manufacturing processes and job categories, respectively. The highest VNA concentrations appeared to occur at production lines producing rubber using *N,N*-dinitrosopentamethylene tetramine as a vulcanization agent. Although there are no numerical occupational *N*-nitrosamine standards in the US, the exposures measured are much higher than the German standard of 1 μg/m^3 total *N*-nitrosamines in general industry and 2.5 μg/m^3 total *N*-nitrosamines for certain processes, such as rubber vulcanization. In Germany, changes in production technology, control and avoidance of raw materials either containing *N*-nitrosamines (*eg N,N*-dinitrosopentamethylene tetramine) or susceptible to nitrosation have reduced occupational exposure to total *N*-nitrosamines to < 0.1 μg/kg body weight/day (Wolf, 1989). In the absence of such measures, exceedingly higher exposure of production workers to *N*-nitrosamines still occurs, as is evident from the NIOSH study (Rey and Fajen, 1996) and recent data from the former USSR showing mean winter levels of 244 ± 75 μg/m^3 NDMA and 836 ± 407 μg/m^3 NDEA in some areas of rubber production, with a mean cumulative NDMA and NDEA exposure of 63 and 1,064 μg/day in summer and winter, respectively (Solionova *et al*, 1992).

Endogenous nitrosation

There is no reason to doubt that amine substrates yield similar nitrosation products endogenously to those formed under *in vitro* conditions by classical nitrosation mechanisms. The acidic gastric compartment would be expected to be the major site of endogenous nitrosation in man. In biological systems, the reactivity and relative stability of *N*- and *S*-containing compounds favours the formation of *N*-nitrosamines and *S*-nitrosothiols compared to alternative *C*- and *O*-nitroso products.

NPRO is formed *in vivo* by the nitrosation of proline (Ohshima and Bartsch, 1981). NTCA and

NMTCA are both formed *in vivo* by condensation of cysteine with formaldehyde or acetaldehyde, respectively, followed by nitrosation (Tricker and Preussmann, 1987). The yields of NTCA and NMTCA are about 250–500 and 60–300 times greater than that of NPRO at pH 1.0–4.0, respectively; optimal formation of NTCA and NMTCA occurs at pH 2.5 and 4.5, respectively, compared to pH 2.5 for NPRO (Ohshima *et al*, 1984). Condensation of homocysteine with formaldehyde and subsequent nitrosation probably results in the formation of *N*-nitrosotetrahydro-4*H*-1,3-thiazine-4-carboxylic acid (NTHTCA) (Nair *et al*, 1986). The secondary amines dimethylamine, pyrrolidine and piperidine, precursors to NDMA, NPYR and NPIP, respectively, are present in nearly all body fluids (Tricker *et al*, 1992). *S*-Nitrosation of thiols, including cysteine and homocysteine (O'Neill and Ohshima, 1987; Stamler *et al*, 1992), and formation of NTCA (Tsuda and Kurashima, 1991a) may provide protection against formation of endogenous *N*-nitrosamines.

Vitamin C (ascorbic acid) and vitamin E (α-tocopherol) both inhibit *in vivo* nitrosation when administered with a nitrostable amine substrate (Ohshima and Bartsch, 1981; Wagner *et al*, 1985). However, in most commercial forms of vitamin E, the tocopherol is esterified and incapable of inhibiting *N*-nitrosation, while the effect of ascorbic acid is only temporal, because of its rapid excretion in urine (Helser *et al*, 1991; Mirvish *et al*, 1995). Only negligible inhibition of NPRO formation occurs under normal dietary conditions after daily supplementation with a 500 mg ascorbic acid tablet, and a capsule containing 100 mg ascorbic acid and 100 IU α-tocopherol each day before breakfast, lunch, dinner and before retiring for the night (Garland *et al*, 1986). This is not totally surprising, since maximal endogenous nitrosation in the gastric compartment appears to occur 1–2 h after meals (Mirvish *et al*, 1995) at which time gastric ascorbate levels are almost depleted. Endogenous formation of *N*-nitrosamines may occur at sites other than the gastric compartment and via different pathways not involving acid-catalysed mechanisms (Hill, 1996).

Cell-mediated nitrosation

Many different mammalian cells produce NO via a common biochemical pathway involving oxidation of the terminal guanido-nitrogen of L-arginine to citrulline by NO synthase (NOS) in the presence of O_2 and NADPH (Förstermann *et al*, 1991; Stuehr *et al*, 1991). A major role for endogenous NO production has been described in virtually every vertebrate organ system involving functions as diverse as bioregulation of vascular tone, platelet aggregation, central and autonomic neurotransmission, immune regulation, bacterial killing, control of airway reactivity and basal airway tone (Änggård, 1994). NOS isoforms are generally classified as either Ca^{2+} dependent and 'constitutive' (cNOS) or Ca^{2+} independent and 'inducible' (iNOS) (Stuehr *et al*, 1991). The Ca^{2+}-mediated calmodulin binding of cNOS isoforms can be stimulated by mediators, including bradykinin, acetylcholine, calcium ionophore, histamine, leukotrienes and platelet activating factor. Isoforms of iNOS are induced by interferon-α, endotoxin, lipopolysaccharide (LPS), tumour necrosis factor (TNF-α, TNF-β), interleukin-1 and a variety of cytokines.

The biological effects of endogenous NO formation depend on a complex interaction of different conditions, including the rate of NO production and its rate of diffusion, the concentrations of potential reactants and necessary enzymes, and the distance between generator and target cells. The half-life of NO in biological systems is estimated to be 0.1 s (Kelm and Schrader, 1990) and is inversely proportional to its concentration (Wink *et al*, 1993). NO can interact directly with cellular guanylate cyclase for signalling (Palmer *et al*, 1987), oxygen species (*eg* superoxide) to form reactive intermediates resembling hydroxyl radicals (Ischiropoulos *et al*, 1992), or molecular oxygen to form nitrosating species (Iyengar *et al*, 1987; Cooney *et al*, 1992). Reactions with water ultimately yield nitrite and nitrate.

Total endogenous cell-mediated mammalian nitrate production under normal conditions amounts to about 1 mmol/day or 1 mg/kg body weight/day in man (Green *et al*, 1981). On a body weight basis, the same order of magnitude is also formed in experimental animals (Leaf *et al*, 1990). Intense physical activity (Leaf *et al*, 1990; Persson *et al*, 1993) and immunostimulation during nonspecific intestinal diarrhoea and fever (Hegesh and Shiloah, 1982) are probably the most important inducers of increased endogenous NO synthesis in otherwise healthy individuals. Immunostimulated NO synthesis occurs primarily in macrophages resulting in the generation of cellular microenvironmental concentrations of NO at a rate of 1 mM/min for several hours (Ischiropoulos *et al*, 1992). Although the production of NO occurs after a lag phase (approximately 4–6 h after stimulation), the amount of NO generated is generally higher than that formed by constitutive processes. The low rates of endogenous NO

production by constitutive processes and activated cells may consequently result in a highly efficient generation of nitrosating species (Tamir *et al*, 1993).

Stimulated macrophages readily nitrosate aliphatic and heterocyclic secondary amines *in vitro* after addition to the medium at physiological pH; the yield of *N*-nitrosamines being partially dependent on amine lipophilicity, and inversely related to basicity (Miwa *et al*, 1987). Stimulated macrophages also nitrosate proline and thiazolidine-4-carboxylic acid (TCA) to yield NPRO and NTCA, respectively (Miwa *et al*, 1989). Addition of nitrite and amines to non-stimulated cells produces negligible yields of *N*-nitrosamines. Compounds generally capable of inhibiting acid-catalysed nitrosation by competing for nitrosating species inhibit nitrosation by macrophages, although ascorbic acid shows a complex behaviour in which nitrosation is enhanced at low ascorbate concentrations (5–50 μM) and inhibited at higher concentrations (Kosaka *et al*, 1989). Inflammatory neutrophil-mediated nitrosation is also inhibited by ascorbic acid (Grisham *et al*, 1992), antioxidants and immunosuppressive agents (Grisham and Miles, 1994).

In rats administered both morpholine and arginine, immunostimulation by treatment with *Escherichia coli* LPS significantly increases excretion of nitrate and *N*-nitroso-(2-hydroxyethyl)glycine (NHEG), a metabolite of endogenously formed NMOR (Leaf *et al*, 1991). Significant formation of NTCA from administered TCA occurs after stimulation of ascorbic acid-deficient mutant rats with *E. coli* LPS (Kosaka *et al*, 1990) and in rats with acute hepatic injury caused by *Propionibacterium acnes* (Wu *et al*, 1993). In the absence of administration of nitrosatable amines, elevated formation of nitrate and NDMA occurs in LPS-stimulated woodchucks with chronic woodchuck hepatitis infection (Lui *et al*, 1991).

Bacterial nitrosation

Several bacterial strains, both denitrifying and non-denitrifying, of the genera *Alcaligenes*, *Bacillus*, *Escherichia*, *Klebsiella*, *Neisseria*, *Proteus* and *Pseudomonas* catalyse the nitrosation of secondary amines (Calmels *et al*, 1985, 1988; O'Donnell *et al*, 1988; Mackerness *et al*, 1989; Charrière *et al*, 1991). Nitrosating activity is linked to the presence of nitrate reductase genes (Calmels *et al*, 1987, 1988; Ralt *et al*, 1988); however, expression of these genes is not always associated with nitrosating activity (Charrière *et al*, 1991). Enterobacteriaceae and Pseudomonadaceae generally express both nitrate reductase and nitrosating activities (Calmels *et al*, 1985; Leach *et al*, 1987; O'Donnell *et al*, 1988; Charrière *et al*, 1991); however, not all strains within the same genus or even the same species catalyse nitrosation (Calmels *et al*, 1985). Nitrosation also depends on the physiological state of the bacteria: *E. coli* catalyses nitrosation of various secondary amines in the resting phase (Calmels *et al*, 1985), while some enteric bacteria (*eg Pseudomonas stutzeri*) catalyse nitrosation only in the growth phase (Mills and Alexander, 1976). Denitrifying bacteria, when suitably induced, show a consistently greater *in vitro* nitrosating capacity than non-denitrifying bacteria (Leach *et al*, 1987). In both denitrifying and non-denitrifying bacteria cytochrome cd_1-nitrite reductase appears to catalyse nitrosation through the production of NO or NO^+-like species (Calmels *et al*, 1996).

Bacterial nitrosation at neutral pH is inhibited by ascorbic acid (Mackerness *et al*, 1989) and cysteine (O'Donnell *et al*, 1988), suggesting that the nitrosating species are most likely N_2O_3 or N_2O_4 produced via an NO intermediate (Ji and Hollocher, 1988); however, the exact mechanism remains unknown (Zumft, 1993). Catalysis of nitrosation follows classical Michaelis–Menton kinetics and does not fit simple first- and second-order kinetics observed for chemical nitrosation of secondary amines (Calmels *et al*, 1985). The specific nitrosation rate is inversely related to the pKa of the amine substrate (Calmels *et al*, 1985); high nitrite concentrations (Calmels *et al*, 1985; Leach *et al*, 1987) and decreasing pH (O'Donnell *et al*, 1988) result in inhibition.

Ambient nitrogen oxides

Inhalation of atmospheric NOx, in particular NO_2, may increase both endogenous nitrate and nitrite exposure as well as the pool of endogenous nitrosating species dissolved in airway lining fluid. Weakly acidic conditions in the airway (Lopez-Vidriero *et al*, 1977) are likely to alter the distribution of dissolved NOx to favour decomposition of nitrite to the nitrosating species N_2O_3 (Ridd, 1978). Dissolved NO_2 present in the lung can react to form nitrous acid by hydrogen abstraction from unsaturated fatty acids, with the initiation of lipid peroxidation (Pryor and Lightsey, 1981), or interact with cholesterol in lung tissues and blood to form cholesteryl-3-β-nitrite, a lipid soluble nitrosating species (Kobayashi and Kubota, 1980).

In the absence of elevated ambient NOx concentrations, pulmonary epithelial cells, macrophages,

neutrophils, mast cells, autonomic neurons, vascular smooth muscle cells, fibroblasts and endothelial cells are all potentially capable of generating NO in the airways via the NOS pathway (Gaston *et al*, 1994), as is evident from the observation that expired air normally contains NO of endogenous origin (Persson *et al*, 1993). The formation of *S*-nitrosothiols in airway lining fluid (Gaston *et al*, 1993) may be an important mechanism mitigating the toxicity derived from reactions of NOx in the lung (Stamler *et al*, 1992).

Animal studies provide only limited evidence that inhalation of NOx can result in endogenous *N*-nitrosamine formation. Gavage of rodents with morpholine followed by exposure to 20–50 ppm NO_2 does not result in significant NMOR formation (Mirvish *et al*, 1981; Van Stee *et al*, 1983), or has produced artifact results during analysis (Iqbal *et al*, 1980). Similarly, NMOR produced in *ex vivo* studies with isolated rat lungs perfused with 10 mM morpholine and ventilated with 20 ppm NO_2 (Postlethwait and Mostafa, 1983) appears to be caused by artifact formation occurring in the lung perfusion apparatus (Cooney *et al*, 1986b). NDMA formation has been reported in rats and rabbits pretreated with aminopyrine, a readily nitrosatable pharmaceutical, and exposed to 100 ppm NO_2 (Uozumi *et al*, 1982). In a more recent study in which mice were given morpholine by gavage and exposed to 20 ppm $^{15}NO_2$, endogenous formation of both ^{14}NMOR and ^{15}NMOR occurred, suggesting that both ambient $^{15}NO_2$ can be converted endogenously to a nitrosating species resulting in the formation of ^{15}NMOR and that ^{14}NMOR is produced via an ongoing mammalian process (Van Stee *et al*, 1995).

Biomonitoring of *N*-nitrosamino acids in urine

The major NVNA found in human urine are NSAR, NPRO, NTCA and NMTCa (Ohshima *et al*, 1984; Tricker and Preussmann, 1987). Excretion of NMPA (Nair *et al*, 1986; Forman *et al*, 1988; Zatonski *et al*, 1989), NHPRO (Tricker and Preussmann, 1987), NAzCA (Nair *et al*, 1986; Zatonski *et al*, 1989) and NTHTCA (Nair *et al*, 1986) has also been reported. Most human biomonitoring studies have compared NSAR, NPRO, NTCA and NMTCA excretion in populations from low- and high-prevalence areas for specific cancers, or healthy control subjects to clinical patient collectives (Table 1). Only those studies are presented in which 24-h urine samples have been collected and excretion calculated as μg/day NVNA. When available, data for the excretion of nitrate is also presented.

Animal studies indicate that almost quantitative urinary excretion (>95%) occurs for NPRO (Chu and Magee, 1981; Ohshima *et al*, 1982), NTCA and NMTCA (Ohshima *et al*, 1984) within 24 h of oral administration, with only trace levels occurring in faeces. NSAR is primarily excreted in urine (87.7 ± 2.4%) with only about 0.07% excretion in faeces, while NHPRO is excreted almost quantitively in both urine (46.8 ± 2.2%) and faeces (46.2 ± 3.2%) (Ohshima *et al*, 1982). Excretion studies have not been reported for other minor NVNA (NMPA, NAzCA and NTHTCA) found in urine.

NSAR and NPRO are chemically stable and easily amenable to gas chromatographic analysis using a thermal energy analyser (Massey, 1988). Depending on the solvent system used, NTCA and NMTCA may be subject to acid-catalysed decomposition during extraction from urine (Lu *et al*, 1986) and thermal degradation may occur upon analysis by gas chromatography. Quantitation may also be impeded by the failure to achieve adequate baseline resolution of NVNA during gas chromatographic separation (Ohshima *et al*, 1984; Tsuda *et al*, 1986). Collaborative studies have not been published to compare interlaboratory variation in the determination of NVNA in urine; therefore, caution must be taken in any cross-study comparisons.

Intraindividual and interindividual variations in urinary NPRO excretion have been studied in 24 h urine samples collected four times per week for 5 weeks from 24 healthy volunteers (Garland *et al*, 1986). During the last 3 weeks of the study, each subject received daily supplementation with a 500 mg ascorbic acid tablet, a capsule containing 100 mg ascorbic acid and 100 IU α-tocopherol each day before breakfast, lunch, dinner and before retiring for the night. The mean NPRO excretion for all subjects was 3.31 ± 4.06 μg/day in the absence of vitamin supplementation and 3.22 ± 4.00 μg/day during supplementation. Urinary NPRO excretion ranged from < 0.20 to 38.1 μg/day (480 samples) for all subjects. The mean urinary excretion of NPRO (20 measurements per subjects) in different individuals ranged from 0.76 ± 0.63 to 9.04 ± 7.41 μg/day. The mean relative standard deviations for interindividual and intraindividual differences were 116% and 84%, respectively. The interindividual variation ranged between 36% and 253%. Similar results were also reported for NTCA excretion which showed a highly significant correlation ($R = 0.74$) with NPRO excretion when the means for each individual were

Table 1. Daily urinary excretion of nitrate and N-nitrosamino acids in population studies[a,b]

Population and comments	*n*	Nitrate (mg/day)	NSAR (μg/day)	NPRO (μg/day)	NTCA (μg/day)	NMTCA (μg/day)	Total (μg/day)	Reference
(West) Germany								
General population	20	97.1 ± 48.1 (20–218)	0.42 ± 0.37 (ND–1.2)	2.20 ± 0.51 (1.4–3.1)	7.24 ± 4.24 (2.3–16.7)	5.08 ± 2.81 (3.6–14.9)	14.43 ± 5.48 (8.9–28.5)	Tricker *et al.* 1989b
General population	24	62.9 ± 29.8 (29.8–105.5)	0.94 ± 0.78 (ND–2.6)	2.84 ± 1.06 (1.2–5.2)	12.04 ± 5.87 (5.0–29.0)	2.84 ± 1.63 (ND–6.2)	18.66 ± 7.84 (8.5–37.2)	Tricker and Eisenbrand, unpublished results
United Kingdom								
Hospital staff	10	89.1 ± 37.4 (30–117)	0.36 ± 0.32 (ND–2.4)	2.15 ± 0.32 (1.6–2.7)	6.92 ± 2.99 (3.9–12.3)	4.68 ± 0.98 (3.5–6.7)	14.07 ± 3.68 (9.7–20.9)	Tricker, 1996
Paraplegic patients	30	47.0 ± 33.4 (1.6–145.9)	0.48 ± 0.36 (ND–2.6)	3.24 ± 0.48[**] (1.9–5.5)	9.32 ± 3.86 (3.2–27.5)	6.02 ± 2.52 (2.8–10.4)	25.16 ± 14.82[*] (13.5–68.4)	
Poland								
Low gastric cancer prevalence	47	90 (7–344)	0.10[#] (0.1–1.2)	2.0 (0.4–19.3)	7.5 (0.1–80.3)	–	11.1[c] (1.5–109)	Zatonski *et al.* 1989
High gastric cancer prevalence	50	96 (36–475)	0.24 (ND–2.4)	1.8 (ND–23.4)	10.5 (ND–23.2)	–	14.6[c] (0.3–243.3)	
Egypt								
Healthy population	27	139.3 ± 82.2 (35–402)	3.40 ± 5.91 (ND–29.6)	7.16 ± 5.07 (1.2–20.1)	11.69 ± 7.60 (3.5–33.4)	9.08 ± 5.55 (3.1–23.2)	31.20 ± 22.07 (9.7–94.8)	Tricker *et al.* 1989b
Schistosomiasis (bilharzial) infection	27	143.6 ± 136.3 (16–506)	6.01 ± 3.45 (ND–13.4)	17.01 ± 7.15[*,‡] (2.9–33.6)	24.37 ± 9.27[*,‡] (3.7–41.4)	15.12 ± 6.81 (1.8–29.2)	62.91 ± 21.96[*,‡] (13.2–108.7)	
Bilharzial bladder cancer	23	175.0 ± 190 (35–855)	6.24 ± 9.90 (2.1–9.9)	12.61 ± 3.37 (2.7–17.2)	15.93 ± 3.78 (9.4–23.7)	9.47 ± 4.60 (4.9–24.1)	44.94 ± 7.31 (24.6–65.5)	
Japan								
General population (men)	19	–	–	1.6 ± 1.1 (0.6–4.5)	6.4 ± 5.5 (0.7–22.9)	5.4 ± 3.6 (0.4–14.5)	13.4 ± 7.2 (2.6–30.9)	Tsuda *et al.* 1987
General population (women)	14	–	–	1.9 ± 0.9 (0.8–3.9)	12.1 ± 5.9[**,‡] (3.0–20.7)	10.2 ± 7.9[**,‡] (1.5–24.7)	24.1 ± 11.4 (5.8–40.6)	
Seventh Day Adventists (men)	18	–	–	4.3 ± 6.0 (0.3–24.4)	5.1 ± 3.4 (1.2–11.7)	3.6 ± 2.9 (0.4–11.7)	13.1 ± 10.8 (2.3–47.2)	
Seventh Day Adventists (women)	18	–	–	3.0 ± 3.2 (0.2–12.6)	5.2 ± 4.8 (0.6–19.4)	3.4 ± 3.7 (0.4–13.8)	11.6 ± 10.0 (1.4–34.2)	
Low gastric cancer prevalence	52	95 [73–117]	–	3.8 [2.6–5.0]	12.4 [7.7–17.1]	3.2 [2.1–4.3]	20.2 [14.7–26.6]	Kamiyama *et al.* 1987
High gastric cancer prevalence	52	145[***] [118–170]	–	6.1 [4.1–8.1]	5.7[*] [2.0–9.4]	2.8 [1.2–4.4]	14.8 [7.4–22.2]	

Table 1 (continued)

Population and comments	*n*	Nitrate (mg/day)	NSAR (μg/day)	NPRO (μg/day)	NTCA (μg/day)	NMTCA (μg/day)	Total (μg/day)	Reference
Low oesophageal cancer prevalence (men)	29	203 ± 111		4.9 ± 4.3	10.3 ± 8.7	5.0 ± 5.6	–	Tuyama *et al.* 1992
Low oesophageal cancer prevalence (women)	28	151 ± 98	–	3.3 ± 3.1	6.6 ± 5.4	1.6 ± 2.0	–	
High oesophageal cancer prevalence (men)	28	167 ± 99	–	3.5 ± 2.3	17.3 ± 15.6	12.3 ± 13.8	–	
High oesophageal cancer prevalence women)	26	218 ± 82	–	4.3 ± 3.1	18.9 ± 15.5	7.5 ± 6.1	–	
Very high oesophageal cancer prevalence (men)	37	166 ± 100	–	4.4 ± 5.5	19.1 ± 21.1	10.0 ± 11.7	–	
Very high oesophageal cancer prevalence (women)	24	187 ± 133	–	5.9 ± 14.3	17.1 ± 19.2	7.3 ± 8.0	–	
China								
Low oesophageal cancer prevalence	40	48 [37–61]	0.1 [0.1–0.18]	1.7 [0.9–2.9]	1.9 [1.6–4.2]	0.1 [0.1–0.94]	5.6 [3.6–8.1]	Lu *et al.* 1986
High oesophageal cancer prevalence	44	94[a,b] [69–119]	0.49[a,b] [0.17–0.63]	5.7[a,b] [4.1–7.7]	13.7[a,b] [8.6–16.9]	0.69 [0.1–0.98]	21.2[a,b] [14.2–25.3]	
Low oesophageal cancer prevalence	44	–	1.5 ± 3.2	2.0 ± 2.6	0.3 ± 1.6	1.3 ± 3.6	6.3 ± 7.2[f]	Lu *et al.* 1987
High oesophageal cancer prevalence	44	–	–	5.7 ± 8.2	2.6 ± 4.9	–	8.7 ± 11.2[f]	
	48	–	2.6 ± 5.7	5.9 ± 9.2	1.9 ± 6.1	0.1 ± 0.8	12.4 ± 16.5[g]	
	54	–	3.8 ± 8.9	4.2 ± 6.3	4.5 ± 6.5	1.0 ± 4.8	21.3 ± 19.9[g]	
	57	–	–	6.6 ± 11.2	2.6 ± 4.3	–	13.7 ± 13.7[g]	
	50	–	1.6 ± 8.5	4.4 ± 6.2	6.7 ± 12.1	0.8 ± 3.8	13.6 ± 18.1[g]	

Abbreviations: NSAR, *N*-nitrososarcosine; NPRO, *N*-nitrosoproline; NTCA, *N*-nitrosothiazolidine-4-carboxylic acid; NMTCA, *N*-nitroso-2-methylthiazolidine-4-carboxylic acid; ND, not detected.

[a]Reported data excludes studies on smokers and nonsmokers (see Table 2). Data reported as either arithmetic means ± SD (range) or geometric means [95% confidence limits].

[b]All tests of significance compare areas of low- and high-prevalence for specific cancers unless otherwise stated: $^{*}P > 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

[c]Total includes 4-(*N*-nitrosomethylamino)propionic acid (NMPA) and NMTCA.

[d]Significance compared to normal population.

[e]Significance compares men to women.

[f]Total includes an unidentified compound (presumed to be NMPA).

considered (Tannenbaum, 1986). Other studies report similar interindividual variations in NSAR, NPRO, NTCA and NMTCA excretion in single subjects (Ohshima *et al*, 1984; Tsuda *et al*, 1986; Tricker and Preussmann, 1987). The influence of sex on NVNA excretion has not been subject to thorough investigation, although some evidence exists to suggest that a sex-related difference in NTCA and NMTCA excretion occurs, with higher excretion rates in women (Tsugane *et al*, 1992). These studies suggest that multiple measurements are required to assess the actual excretion status of an individual.

Effect of diet

The lowest mean excretion of NPRO (about 0.3 μg/day) has been reported in Indian subjects (Nair *et al*, 1985, 1986; Chakradeo *et al*, 1994); considerably lower than that reported in other populations (Table 1). The low level of NPRO excretion may be a result of the mainly vegetarian Indian diet, which lacks nitrate-preserved foodstuffs. A low NPRO excretion is observed in Canadian vegetarians (0.80 ± 0.93 μg/day) and lacto-vegetarians (1.37 ± 1.49 μg/day) (Stich *et al*, 1984). Swedish lacto-vegetarians also have a low NPRO excretion (1.33 ± 0.94 μg/day) compared with subjects eating meat-containing diets (2.30 ± 2.12 μg/day) (Halling *et al*, 1989). Changing from eating meat-containing diets to vegetarian diets results in significant sequential daily reductions in NPRO excretion (Stich *et al*, 1984).

Canadian Inuits consuming 'western' diets containing nitrate-preserved foods have significantly increased excretion of NPRO ($P < 0.05$) compared to Inuits with traditional fresh meat and fish diets (Stich and Hornby, 1988). Consumption of nitrate-cured meat products is also associated with increased urinary NPRO excretion in Denmark (Moller *et al*, 1989). More than 90% of NPRO in meat products is protein-bound and not amenable to analysis without prior hydrolytic or enzymatic digestion (Dunn and Stich, 1984; Sen *et al*, 1989), consumption of which provides a significant source of urinary NPRO both in man (Stich *et al*, 1984) and experimental animals (Perciballi *et al*, 1989; Sen *et al*, 1989). In ferrets, protein-bound NPRO in diets containing cured meat accounts for about 70% of urinary NPRO excretion, while < 17% is formed endogenously (Perciballi *et al*, 1989).

Dietary fish significantly increases NTCA excretion (Tsuda *et al*, 1988); consumption of Tara-chiri, a traditional Japanese dish containing cod and radish, significantly increases ($P < 0.001$) NTCA excretion from 7.9 ± 4.2 to 110 ± 64.5 μg/day. Other cod-based diets may increase NTCA excretion to as much as 300 μg/12 h. The cause of the increased excretion of NTCA appears to be due to endogenous nitrosation of relatively high concentrations of TCA (about 4.0 mg/kg) present in fish.

Other dietary factors have not been extensively studied in individual subjects or populations. The effects of diet on the urinary excretion of NPRO and NTCA suggest that in the absence of a modest degree of dietary control, comparisons of NVNA excretion in small population groups may be significantly influenced by a small number of individuals with dietary habits deviating from the majority of the population group.

Effect of nitrate

Dietary supplementation with 217 mg [^{15}N]-nitrate results in a 5- to 6-fold increase in NPRO and NTCA excretion; only about 10–60% of urinary NPRO contains the ^{15}N label and the incorporation of [^{15}N]-nitrate into NPRO, but not NTCA, is inhibited by both ascorbic acid and α-tocopherol (Wagner *et al*, 1985). Higher levels of nitrate supplementation (600 mg/day) significantly increases urinary excretion of NSAR (0.43 ± 0.37 *vs* 0.98 ± 0.57 μg/day; $P < 0.05$), NPRO (2.09 ± 0.60 *vs* 15.3 ± 5.9 μg/day; $P < 0.05$), NTCA (4.93 ± 3.01 *vs* 34.8 ± 28.8 μg/day; $P < 0.05$), NMTCA (6.83 ± 3.48 *vs* 77.0 ± 56.6 μg/day; $P < 0.05$), NHPRO (1.44 ± 0.45 *vs* 16.2 ± 9.2 μg/day; $P < 0.05$) and total NVNA excretion (15.4 ± 5.9 *vs* 159.8 ± 84.6 μg/day; $P < 0.05$) (Tricker and Preussmann, 1987). After intravenous administration of 5.56–8.35 g/day nitrate as NH_4NO_3 to healthy adult volunteers, maximum urinary NPRO excretion increases to 320 μg/l (Ellen and Schuller, 1984).

Under normal dietary conditions, a strong correlation ($P = 0.002$) is reported between intake of nitrate in drinking water and urinary NPRO excretion in rural Nebraska (Mirvish *et al*, 1992). Similarly, nitrate intake from drinking water is strongly associated with NPRO excretion in rural Danish non-smokers (χ^2 test for trend; $P = 0.08$), but not in smokers (Møller *et al*, 1989). A significant correlation between urinary nitrate and NPRO excretion ($R = 0.297$; $P = 0.0001$) has also been reported for Colombians living in a high-prevalence area for gastric cancer (Stillwell *et al*, 1991a). Nonsignificant but positive trends between urinary nitrate and NPRO excretion are found in both Italian (Knight *et al*, 1992) and Polish (Zatonski *et al*, 1989) subjects from different areas with

contrasting rates of gastric cancer. Contradictory results show both a correlation (Tsugane *et al*, 1992) or no correlation (Kamiyama *et al*, 1987) between urinary nitrate and NPRO excretion in Japanese populations living in high-prevalence areas for gastric cancer. Chinese studies show significant correlations between urinary nitrate and NPRO excretion in subjects ($R = 0.47$; $P = 0.0001$) living in low- and high-prevalence areas for nasopharyngeal cancer in southern China (Zeng *et al*, 1993) and in subjects ($R = 0.39$; $P < 0.01$) living in low- and high-prevalence areas for oesophageal cancer in northern China (Lu *et al*, 1986).

Effect of smoking

Mechanisms by which smoking may influence NVNA excretion have been reviewed (Tsuda and Kurashima, 1991b). Cigarette smoke contains NOx (mainly in the form of NO (Williams, 1980; Norman *et al*, 1983)), formaldehyde and acetaldehyde (Houlgate *et al*, 1989). It is speculated that condensation of these aldehydes, which are present in tobacco smoke, with cysteine in airway lining fluid produces nitrosatable precursors to NTCA and NMTCA; however, studies in which mainstream cigarette smoke is passed into phosphate buffer (pH 7.4) containing cysteine indicate that only negligible amounts of NMTCA are formed (Tsuda and Kurashima, 1991b). Cigarette smoke also contains hydrogen cyanide (Norman *et al*, 1983) and acrylonitrile (Grob, 1962), both of which are metabolized to thiocyanate (Fiedler and Wood, 1956; Lambotte-Vandepaer *et al*, 1985), an effective catalyst of nitrosation *in vitro* (Fine and Tannenbaum, 1973) and *in vivo* in experimental animals (Ohshima *et al*, 1982; Licht *et al*, 1988b).

Several studies have compared the daily excretion of NVNA in smokers and nonsmokers (Table 2). Under experimentally controlled dietary conditions smoking significantly increases ($P < 0.05$) NPRO excretion (Hoffmann and Brunnemann, 1983); however, when dietary restrictions are used to prevent ingestion of significant levels of preformed NPRO and inhibitors of endogenous NPRO formation, only a slight, but not significant, increase is observed in smokers (Ladd *et al*, 1984). In a controlled diet study in which a male volunteer smoked 20 cigarettes/day for 20 nonconsecutive days and did not smoke for 20 nonconsecutive days during a 4-month period, increased urinary excretion of NPRO ($P < 0.01$), NTCA ($P < 0.001$), NMTCA ($P < 0.01$) and total NVNA ($P < 0.001$) occurred during the smoking period (Tsuda *et al*, 1986); however, the increase was not significantly different to that observed during consumption of an unrestricted diet (Tsuda *et al*, 1987). Urinary excretion of NTCA and NMTCA was also reported to be significantly higher in male smokers compared with both female smokers and male nonsmokers; the influence of sex on NTCA and NMTCA excretion was more significant than that of smoking. According to an Italian study, smoking does not affect the urinary excretion of NPRO; however, total NVNA (NSAR, NPRO, NTCA and NMTCA) excretion significantly increased in smokers of blond tobacco ($P < 0.05$) and black tobacco ($P < 0.05$), but not in smokers of both types of tobacco, when compared with nonsmokers (Malaveille *et al*, 1989). After correction for urinary creatinine, statistically significant positive dose–effect relationships were observed between urinary nicotine plus cotinine with NPRO ($P < 0.05$) and total NVNA ($P < 0.01$) excretion (Malaveille *et al*, 1989). Other studies have reported no significant effect of smoking on the excretion of NVNA (Ohshima *et al*, 1984; Cooney *et al*, 1986a; Nair *et al*, 1986; Knight *et al*, 1991; You *et al*, 1996).

In population studies performed in low- and high-prevalence areas for gastric cancer in which smoking was also taken into consideration, its effect on urinary NVNA excretion is even less clear. In agreement with the above Italian study (Malaveille *et al*, 1989), smoking had no effect on urinary NPRO excretion in two separate Italian studies (Knight *et al*, 1991, 1992). No significant effect of smoking was found on NPRO excretion in Polish subjects; however, smokers had a tendency for higher excretion of NMPA and NMTCA with increasing number of cigarettes smoked (Zatonski *et al*, 1989). In Japanese subjects, no effect of smoking was found on NPRO and NMTCA excretion while a significant increase ($P < 0.005$) in NTCA excretion was observed (Kamiyama *et al*, 1987). Contrary to all these observations, NPRO excretion is lower in Colombian smokers compared with nonsmokers (Stillwell *et al*, 1991b).

Contradictory results have been reported on the effect of smoking in four Chinese population studies performed by the same working group. No significant effect of smoking was observed on urinary NSAR, NPRO, NTCA, NMTCA and total NVNA excretion in two representative subgroups of 50 current smokers and 33 nonsmokers from a study population of 538 subjects (Chang-Claude *et al*, 1991), or in all of 4,000 subjects (Wu *et al*, 1993), from a high-prevalence area for oesophageal cancer.

Table 2. Daily urinary excretion of nitrate and *N*-nitrosamino acids in healthy nonsmokers and smokers[a,b]

Population and comments	*n*	Nitrate (mg day)	NSAR (μg day)	NPRO (μg day)	NTCA (μg day)	NMTCA (μg day)	Total (μg day)	Reference
(West) Germany								
Nonsmokers	10	93.7 ± 45.4 (41–195)	0.39 ± 0.34 (ND–1.0)	2.09 ± 0.44 (1.4–2.6)	5.79 ± 3.45 (3.3–12.6)	5.03 ± 1.53 (3.6–8.6)	13.33 ± 4.64 (8.9–24.4)	Tricker *et al.* 1989
Smokers	10	106.8 ± 54.8 (30–218)	0.52 ± 0.44 (ND–1.2)	2.28 ± 0.57 (1.4–3.1)	9.07 ± 4.09 (4.8–16.7)	7.22 ± 3.46 (4.2–14.9)	19.09 ± 6.49 (12.0–28.5)	
France								
Nonsmokers	18	–	0.27 ± 0.31 (ND–0.99)	4.12 ± 4.52 (0.9–14.3)	5.93 ± 5.91 (0.9–21.4)	3.09 ± 3.57 (0.4–10.5)	13.45 ± 11.84 (3.9–34.4)	Oshima *et al.* 1984
Smokers	8	–	0.54 ± 0.58 (ND–1.6)	4.36 ± 3.71 (1.1–12.4)	14.49 ± 13.34 (3.1–35.0)	7.41 ± 6.10 (1.0–19.8)	26.91 ± 18.95[c] (7.2–53.8)	
Nonsmokers	7	–	0.27 ± 0.34 (ND–0.9)	4.49 ± 4.21 (0.9–10.5)	8.56 ± 7.00 (1.7–21.4)	2.43 ± 3.60 (0.6–10.5)	16.09 ± 11.84 (3.9–34.4)	Nair *et al.* 1986
Smokers	7	–	0.73 ± 1.08 (ND–3.0)	5.87 ± 3.65 (1.6–12.4)	17.29 ± 13.12 (3.1–35.0)	6.27 ± 6.34 (1.0–19.8)	30.89 ± 18.88 (7.2–53.8)	
Italy								
Nonsmokers	32	75.50 ± 45.77 (19–173)		2.19 ± 1.18 (0.5–5.4)	–	–	10.59 ± 6.17[c] (1.7–26.3)	Malaveille *et al.* 1989
Smokers of blond tobacco	48	89.92 ± 50.82 (18–232)		2.48 ± 1.29 (0.5–5.5)	–	–	14.99 ± 10.49[c] (2.6–51.0)	
Smokers of black tobacco	15	79.73 ± 26.35 (27–134)		2.99 ± 1.79 (0.7–5.8)	–	–	16.85 ± 11.01[c] (1.9–39.5)	
Smokers of mixed tobacco	9	69.44 ± 41.49 (33–167)		1.57 ± 0.82 (0.6–3.4)	–	–	10.09 ± 7.05[c] (4.6–27.4)	
All smokers	72	85.24 ± 45.75 (21–232)		2.48 ± 1.42 (0.5–5.8)	–	–	14.76 ± 10.30[c] (1.9–51.0)	

Table 2. (Continued)

Population and comment	*n*	Nitrate (mg/day)	NSAR (μg/day)	NPRO (μg/day)	NTCA (μg/day)	NMTCA (μg/day)	Total (μg/day)	Reference
Japan								
Nonsmoking men	19	–	–	1.6 ± 1.1 (0.6–4.5)	6.4 ± 5.5 (0.7–22.9)	5.4 ± 3.6 (0.4–14.5)	13.4 ± 7.2 (2.0–30.9)	Tsuda *et al.* 1987
Nonsmoking women	20	–	–	1.9 ± 0.9 (0.8–3.9)	12.1 ± 5.9[**d] (3.0–20.7)	10.2 ± 7.9[**d] (1.5–24.7)	24.1 ± 11.4 (5.8–40.6)	
Smoking men	17	–	–	2.2 ± 1.2 (0.8–3.9)	10.5 ± 6.5[*d] (3.6–23.1)	8.8 ± 4.1[*d] (1.4–14.9)	24.2 ± 11.4 (5.8–40.6)	
Abstinent male smoker on normal diet	14	–	–	1.4 ± 0.8 (0.7–3.6)	7.9 ± 6.4[**e] (2.2–24.9)	8.7 ± 6.4[**e] (1.1–27.6)	18.0 ± 12.4 (4.6–48.9)	Tsuda *et al.* 1986, 1987
Abstinent male smoker on 20 day fixed diet	20	–	–	1.1 ± 0.5 (0.3–2.6)	3.9 ± 1.1 (1.8–6.0)	5.6 ± 1.9 (2.4–9.2)	10.6 ± 2.8 (5.9–14.7)	
Male smoker on 20 day fixed diet	20	–	–	1.8 ± 0.9[**e] (0.4–4.1)	8.7 ± 4.6[**e] (2.7–21.3)	8.5 ± 4.1[**e] (2.9–19.0)	19.0 ± 6.5[***e] (9.1–28.5)	

Abbreviations: NSAR, *N*-nitrososarcosine; NPRO, *N*-nitrosoproline; NTCA, *N*-nitrosothiazoline-4-carboxylic acid; NMTCA, *N*-nitroso-2-methylthiazolidine-4-carboxylic acid; ND, not detected.

[a]Reported data excludes population studies in areas with low- and high-prevalence for specific cancer (see Table 1). Data reported as arithmetic means ± SD.

[b]All tests of significance compare nonsmokers to smokers unless otherwise stated: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

[c]Total includes NSAR, NTCA and NMTCA.

[d]Significance compared to nonsmoking men

[e]Significance compared to abstinent male smoker on fixed diet.

In two smaller studies, smokers had a tendency for higher excretion of NPRO, NTCA, NMTCA and total NVNA ($P < 0.01$) in low- and high-prevalence areas for oesophageal cancer in northern China (Lu *et al*, 1986), and significantly higher excretion of NPRO ($P < 0.01$), NTCA ($P < 0.01$) and total NVNA ($P < 0.001$) than nonsmokers in low- and high-prevalence areas for nasopharyngeal cancer in southern China (Zeng *et al*, 1993).

Effect of infection

Infection with *Opisthorchis viverrini* (liver fluke) in northeast Thailand is claimed to increase basal NPRO excretion from 4.80 ± 0.80 to 12.37 ± 3.44 µg/day (Srianujata *et al*, 1987). Although hepatic *O. viverrini* infection results in stimulation of NOS (Srivatanakul *et al*, 1991), some doubt exists as to whether the higher NPRO levels observed in infected subjects is a result of an increased potential for endogenous nitrosation (Haswell-Elkins *et al*, 1994). *Schistosomiasis* infection (bilharzia) in Egypt significantly increases NVNA excretion (Tricker *et al*, 1989b). Bacterial infection of the urinary bladder increases urinary excretion of NPRO (2.25 ± 3.64 *vs* 0.94 ± 0.81 µg/l), NTCA (8.26 ± 20.22 *vs* 1.91 ± 3.14 µg/l) and NMTCA (1.64 ± 7.66 *vs* 0.25 ± 0.74 µg/l), and significantly increases nitrite excretion from nondetected levels to 2.16 ± 3.63 (range ND–15.2) mg/l (Ohshima *et al*, 1987). Bacterial infection of the bladder is nearly always associated with an increase in NVNA excretion and urinary excretion of nitrite. Paraplegic patients with bacterial infection of the bladder have significantly ($P < 0.05$) increased excretion of total NVNA compared with healthy subjects (Tricker, 1996).

Urinary diversion in which the bladder is resected and the ureters implanted into the sigmoid colon (ureterosigmoidostomy) results in direct contact of urinary amines and nitrate with the faecal bacterial flora. In comparison to normal bladder urines, rectal urine samples from ureterosigmoidostomy patients contain significantly less nitrate (58.7 ± 24.2 *vs* 16.7 ± 14.3 mg/l; $P < 0.0001$), more nitrite (ND *vs* 1.35 ± 1.68 mg/l; $P < 0.0001$), significantly higher levels of NSAR (0.93 ± 0.92 *vs* 0.27 ± 0.28 µg/l; $P < 0.02$), NPRO (3.11 ± 2.63 *vs* 1.40 ± 0.45 µg/l; $P < 0.001$) and NTCA (6.51 ± 4.49 *vs* 4.04 ± 2.65 µg/l; $P < 0.05$), and significantly less NMTCA (2.45 ± 3.07 *vs* 3.55 ± 3.28 µg/l; $P < 0.05$) (Tricker *et al*, 1989c). Daily administration of 2 g ascorbic acid (1 g in children) does not significantly reduce NVNA and nitrite excretion in ureterosigmoidostomy patients (Kälble *et al*, 1991).

Biomonitoring of volatile *N*-nitrosamines in urine

A mean basal excretion of 34.8 ± 28.8 (range < 5–190) ng/day NDMA has been reported in 24 healthy subjects studied four times per week for 5 weeks (Garland *et al*, 1986). NDMA excretion correlated significantly ($R = 0.69$; $P < 0.005$) with atmospheric NO_x levels. The mean intraindividual urinary excretion (20 measurements per subject) ranged from 22.4 ± 10.9 to 75.4 ± 35.0 ng/day NDMA, and was not affected by daily supplementation with 900 mg vitamin C and 400 IU vitamin E during the last 2 weeks of the study. The mean relative standard deviation for interindividual and intraindividual differences were 52% and 51%, respectively. On an individual basis, the intraindividual variation ranged between 37% and 86%. The mean urinary excretion of 24.6 ± 14.0 pg/ml NDMA (480 24 h urine samples) agrees well with that of 25.8 ± 16.4 (range ND–65) pg/ml NDMA found in a smaller study (Webb *et al*, 1979).

The very low levels of NDMA excretion in healthy subjects is supported by animal studies in which hepatic first-pass metabolism of low doses of NDMA precludes significant excretion in urine (Swann *et al*, 1984; Anderson *et al*, 1992). In the rat, oral doses of 50 or 350 µg NDMA/rat results in only 0.02 or 0.03% excretion in urine, respectively (Spiegelhalder *et al*, 1982). VNA present in the urinary bladder may be metabolized by urothelial cells (Airoldi *et al*, 1992) or absorbed into the blood stream, resulting in increased systemic exposure (Hawksworth and Hill, 1974). As a consequence, determination of urinary VNA does not reflect exogenous exposure or endogenous formation.

Effect of alcohol and smoking

Basal NDMA excretion is significantly increased in nonsmoking drinkers (41.3 ± 22.2 ng/day; $P < 0.01$) and smoking drinkers (65.0 ± 31.6 ng/day; $P < 0.001$) compared with subjects who do not drink or smoke (32.2 ± 21.8 ng/day) (Cooney *et al*, 1986a). Smoking alone significantly increases ($P < 0.0001$) NDMA excretion to 53.3 ± 26.8 ng/day. Experimental studies show that consumption of 500 ml beer containing 12–30 µg NDMA results in 0.5–1.3% excretion of NDMA in urine; when the same NDMA dose was administered in orange juice, excretion of NDMA was only observed after fortification of orange juice with 6% ethanol (Spiegelhalder *et al*, 1982). Other studies have failed to detect NDMA excretion in fasting subjects after

consumption of alcohol (Milligan *et al*, 1986). However, the limit of detection in both studies (2.5 ng/ml NDMA) would not have been sensitive enough to detect the basal NDMA excretion reported in other studies (Webb *et al*, 1979; Cooney *et al*, 1986a; Garland *et al*, 1986).

Effect of infection

Endogenous nitrosation caused by bacterial infection of the urinary tract can result in excretion of 0.94 ± 2.42 (range ND–11.84) μg/l NDMA, 0.5 ± 1.4 (range ND–6.2) μg/l NPYR and 0.17 ± 0.35 (range ND–1.68) μg/l NPIP (Ohshima *et al*, 1987). In spine-injured paraplegic patients with bacterial infection of the urinary tract, mean daily excretion of 0.65 ± 0.69 (range ND–2.68) μg/day NDMA, 0.39 ± 0.50 (range ND–1.96) μg/day NPYR, 0.25 ± 0.44 (range ND–2.13) μg/day NPIP and 10.4 ± 13.2 (range ND–45.9) mg/day nitrite are observed (Tricker *et al*, 1991c).

Concurrent *Schistosomiasis* infection by *S. haematobium* and *S. mansoni* in Egypt is associated with excretion of 2.74 ± 6.13 μg/day NDMA and significantly increased excretion of 0.26 ± 0.35 μg/day NPYR ($P < 0.02$), 0.21 ± 0.43 μg/day NPIP ($P < 0.05$) and 5.18 ± 9.11 mg/day nitrite ($P < 0.02$) compared with subjects without infection (Tricker *et al*, 1989b). Compared with *S. mansoni* infection, *S. haematobium* infection significantly increases excretion of NDMA (19.2 ± 21.0 *vs* 2.9 ± 2.9 μg/day), NPYR (1.3 ± 1.9 *vs* 0.9 ± 0.9 μg/day) and NPIP (1.6 ± 2.3 *vs* 0.4 ± 0.3 μg/day) (Mostaffa *et al*, 1994). Nitrate excretion is also significantly higher after infection by either *S. mansoni* (249 ± 126 mg/day; $P < 0.001$) or *S. haematobium* (174 ± 176 mg/day; $P < 0.005$) compared with subjects without infection (139 ± 82 mg/day), while nitrite excretion shows a reverse trend with higher levels of excretion after infection by *S. haematobium* compared with *S. mansoni* (7.9 ± 24 *vs* 3.2 ± 13 mg/day). Excretion of VNA and nitrite persists in subjects with confirmed *Schistosomiasis* infection and diagnosed bilharzial bladder cancer (Tricker *et al*, 1989b).

In comparison with normal bladder urine, rectal urine from ureterosigmoidostomy patients contains significant levels of 0.37 ± 0.35 μg/l NDMA ($P < 0.0001$), 0.20 ± 0.27 μg/l NPYR ($P < 0.001$) and 0.09 ± 0.17 μg/l NPIP ($P < 0.01$) (Tricker *et al*, 1989c). As in the case of NVNA excretion, daily treatment with 2 g ascorbic acid (1 g in children) does not significantly reduce VNA excretion (Kälble *et al*, 1991). Alternative forms of urinary diversion via an ileal reservoir (Tricker *et al*, 1989c; Gröschel *et al*, 1992) or a Gersuny bladder (Tricker *et al*, 1989c), in which direct contact of urine with faeces is avoided, are not effective in reducing VNA excretion caused by recurrent bacterial infection.

Biomonitoring of urinary *N*-nitrosamines from other sources

Tobacco and tobacco smoke

The major metabolic pathway of NNK in cultured human tissues involves carbonyl reduction to enantiomeric NNAL (Hecht, 1994) followed by *O*-glucuronidation to diastereomeric conjugates of [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-*O*-D-glucosiduronic acid (NNAL-Gluc) (Murphy *et al*, 1994). Both NNAL and NNAL-Gluc are present in smokers' urine (Table 3) and account for 39–101% of the estimated uptake of NNK (Carmella *et al*, 1993). NNAL-Gluc is assumed to be a urinary detoxification product of NNK metabolism (Hecht, 1994) and total excretion of NNAL plus NNAL-Gluc is considerably influenced by dietary factors (Hecht *et al*, 1995). Intraindividual differences in the NNAL-Gluc:NNAL excretion ratio are generally small (< 10%), whereas 16-fold interindividual differences occur in which two phenotypes of this ratio are apparent (Carmella *et al*, 1995). Strong correlations are observed between urinary NNAL, NNAL-Gluc, and NNAL plus NNAL-Gluc with cotinine excretion ($n = 61$; $R = 0.58$; $P < 0.0001$), but not with the number of cigarettes smoked per day ($R = 0.05$) (Carmella *et al*, 1995). Both NNK metabolites also occur in urine of pipe smokers (B. Pfundstein, personal communication).

Considerably higher urinary excretion of 81 ± 30 (range 43.9–129.7) μg/l NNAL and 341 ± 195 (range 138.9–579.2) μg/l NNAL-Gluc occurs in Sudanese snuff-dippers using toombak tobacco (Murphy *et al*, 1994). Total NNAL plus NNAL-Gluc excretion also correlates ($R = 0.94$; $P < 0.05$) with urinary cotinine. Since toombak tobacco contains both preformed NNK and, to a less extent, NNAL (Prokopczyk *et al*, 1995), excretion of NNAL plus NNAL-Gluc is not a specific biomarker for NNK uptake in toombak users. NNAL and NNAL-Gluc excretion have also been reported in American snuff-dippers and tobacco chewers; however, unlike the situation in smokers, there was no indication of two phenotypes of the NNAL-Gluc:NNAL excretion ratio (Kresty *et al*, 1996). Nasal snuff use also results in urinary excretion of NNAL plus NNAL-Gluc (B. Pfundstein, personal

Table 3. Daily urinary excretion of 4-(methylnitrosamino)-3-(1-pyridyl)-1-butanone (NNK) metabolites in urine of healthy nonsmokers and smokers

Smoking status	*n*	Cotinine (mg/day)	Urinary excretion of NNK metabolites (μg/day) NNAL[a]	NNAL-Gluc[b]	NNAL-Gluc/NNAL ratio[c]	Reference
Nonsmokers	7		ND	ND		Carmella *et al.* 1993
Smokers (23.3 ± 6.8; range 12–36 cigarettes/day)	11	7.73 ± 5.47 (2.28–20.0)	0.52 ± 0.26 (0.23–1.0)	3.47 ± 1.77 (0.57–6.5)	0.5–10.0	
Smokers (13.9 ± 3.5; range 8–17 cigarettes/day)	9	7.31 ± 1.72 (4.49–10.1)	0.16 ± 0.10 (0.04–0.29)	1.07 ± 0.67 (0.20–1.77)	2.4–4.6	Carmella *et al.* 1995
Smokers (14.8 ± 3.4 cigarettes/day)	33	5.83 ± 2.50	0.15 ± 0.10	1.00 ± 0.55		Hecht *et al.* 1995
Smokers (20.9 ± 4.6; range 8–30 cigarettes/day)	19	4.72 ± 2.32 (0.59–8.72)	0.31 ± 0.24 (0.05–0.94)	0.63 ± 0.36 (0.15–3.10)	0.5 ± 4.9	Meger *et al.* 1996

Abbreviations: NNAL, 4-(methylnitrosamino)-3-(1-pyridyl)-1-butanol; NNAL-Gluc, [4-(methylnitrosamino)-3-(1-pyridyl)but-1-yl]-β-*O*-D-glucosiduronic acid.
[a]To convert to nmol/day multiply by 4.78.
[b]To convert to nmol/day multiply by 2.59.
[c]Calculated on a molar basis.

communication). These results indicate that NNK is metabolized and excreted in the urine of smokers and tobacco users as NNAL and NNAL-Gluc, regardless of the route of uptake.

In an experimental study, excretion of 33.9 ± 20.0 ng/day NNAL plus NNAL-Gluc was observed after 90 min exposure to sidestream cigarette smoke containing 75–263 ng/m^3 NNK generated by machine-smoked reference cigarettes (Hecht *et al.* 1993). Although the experimental conditions in which sidestream cigarette smoke was used as a surrogate for ETS were claimed to be comparable to those in a heavily smoke-polluted bar, the same authors (Brunnemann *et al.* 1992) and others (Klus *et al.* 1992; Tricker *et al.* 1994) report 10- to 20-fold lower concentrations of NNK in ETS. Real-life exposure of nonsmokers to ETS would, therefore, not be expected to result in significant amounts of NNAL and/or NNAL-Gluc excretion.

Excretion of 44–163 ng/day iso-NNAC has been reported in four of 19 smokers and not in non-smokers (Tricker *et al.* 1993). The very low levels of iso-NNAC in mainstream cigarette smoke (< 5 ng/cigarette) (Djordjevic *et al.* 1991; Tricker *et al.* 1993) explain why only low levels of iso-NNAC are occasionally found in urine from some smokers.

Cosmetic products

In an experimental study using a single adult male volunteer, application of a facial cosmetic foundation to the back (12.7 g containing 980 μg NDELA) and removal 7.75 h later resulted in a total cumulative urinary excretion of at least 17.3 μg NDELA over a 21.5 h period (one urine fraction was lost for analysis) (Edwards *et al.* 1979). Although this study indicates that dermal absorption and excretion of NDELA occurs in man, normal use of cosmetic products is very unlikely to result in sufficient exposure NDELA to allow its detection in urine (European Chemical Industry Ecology and Toxicology Center, 1990).

Pharmaceutical products

Several hundred pharmaceutical drugs contain secondary and tertiary amino groups potentially susceptible to endogenous nitrosation to yield NDMA and NDEA, and to a lesser extent, NPYR, NPIP, NMOR and *N*-nitrosohexamethylenimine (Lijinsky, 1990). Standardized *in vitro* nitrosation assays performed under simulated gastric conditions (Coulston and Dunne, 1980; Lijinsky, 1990) indicate that several pharmaceutical drugs may react to produce *N*-nitrosamines *in vivo*; however, only in a few cases has confirmation been obtained in man.

Piperazine is readily nitrosated *in vitro* at pH 0.5–5.5 to yield NMPz, and to a lesser extent, *N,N*-dinitrosopiperazine (DNPz) (Mirvish, 1975; Tricker *et al*, 1991d). Extensive metabolism of NMPz occurs in the rat resulting in the excretion of *N*-nitroso-hydroxypyrrolidine (NHPYR) and NDELA (Tricker *et al*, 1991e). Consequently, patients treated with 2 g/day piperazine (contaminated with 3.6 μg MNPz/g piperazine) have been shown to have a mean post-administration excretion of 27.0 ± 26.7 (range 0.6–96.0) μg/day MNPz, 0.73 ± 0.92 (range ND–2.8) μg/day DNPz, 1.74 ± 1.72 (range ND–5.7) μg/day NHPYR, and < 0.3 μg/day NDELA (Tricker *et al*, 1991d). In a follow-up experimental study, dietary supplementation with 250 mg/day nitrate increased the mean MNPz excretion from 36.9 ± 34.1 (range 92.–80.1) to 84.1 ± 57.7 (range 63.0–122.7) μg/day in five human volunteers receiving a therapeutic dose of 1.9 g piperazine (contaminated with 4.2 μg MNPz/g piperazine) (Kumar *et al*, 1992). Other experimental studies have shown that administration of 480 mg piperazine in syrup to fasting volunteers results in gastric juice concentrations of 140–230 μg/l MNPz after 30 min, with detectable levels still present at 150 min (Bellander *et al*, 1985). Although partial inhibition of endogenous nitrosation was achieved by co-administration of ascorbic acid, no correlation was found between gastric pH and MNPz formation. Urinary *N*-nitroso compounds derived from the endogenous nitrosation of piperazine probably only represent about 10% of the total level of endogenous formation (Bellander, 1990).

Experimental studies show that the analgesic aminopyrene is readily nitrosated *in vivo* (Spiegelhalder and Preussmann, 1985). Following administration of 500 mg aminopyrene, 40 g ethanol as beer and 250 mg nitrate in radish juice, 8 h urinary excretion of 0.5–10 μg NDMA was observed. The total endogenous formation was estimated to be 25–1,800 μg NDMA based on previous experimental studies on NDMA excretion in man (Spiegelhalder *et al*, 1982) and was only apparent after ethanol consumption to inhibit hepatic metabolism. Similarly, administration of the analgesic metamizol (500 mg) results in excretion of 370 μg/day *N*-nitrosomethylaminoantipyrine, the formation of which is not inhibited by co-administration of ascorbic acid (Ziebarth, 1994). Administration of the antibiotic oxytetracycline (500 mg every 8 h) to patients with bacterial infection of the urinary bladder has also been reported to result in excretion of < 0.16 μg/l NDMA (Webb *et al*, 1983); however, this observation requires confirmation.

Occupational exposure

Machine workers handling NDELA contaminated products can be exposed to NDELA by direct contact or by inhalation of oil mists during most machine shop operations. Excretion of NDELA in exposed workers increases progressively over the course of the working week and correlates with the level of NDELA contamination present in the cutting fluids currently being used (Spiegelhalder *et al*, 1984). In Germany, reductions in NDELA contamination of commercial synthetic cutting fluids are reflected in the observed fall in urinary excretion of NDELA from maximum levels of 103 μg/l urine in 1984 (Spiegelhalder *et al*, 1984) to 0.6 ± 1.6 (range ND–15.0) μg/l in 1992 (Bolm-Audorff *et al*, 1992). At the time of urine collection in 1992, cutting fluids present in the studied workshops contained a mean of 20.6 ± 5.0 (range 2–135) mg/l NDELA resulting in ambient air concentrations of 214 ± 391 (range < 50–1,000) ng/m^3 (Fuchs *et al*, 1995).

Occupational exposure of pharmaceutical production workers to piperazine dust results in endogenous formation and excretion of 0.3–4.7 μg/day MNPz (Bellander *et al*, 1988). NMPz excretion correlates significantly with both breathing zone exposure to piperazine dust (0.06–1.7 mg/m^3; $R = 0.78$; $P = 0.01$) and urinary piperazine excretion (0.1–4.7 mg/day; $R = 0.86$; $P = 0.003$); but not with salivary nitrate and nitrite, or urinary nitrate excretion.

Assessment of endogenous nitrosation by the *N*-nitrosoproline test

The *N*-nitrosoproline test in humans involves sequential administration of nitrate and L-proline to volunteers and determination of endogenous formation of NPRO which is rapidly and quantitatively excreted in urine over the next 24 h (Ohshima and Bartsch, 1981). NPRO excretion is proportional to the proline dose, increases exponentially at a nitrate dose above 186 mg, and can be partially blocked by co-administration of high doses (> 1 g) of ascorbic acid. Several modifications to the original administration protocol (Ohshima and Bartsch, 1981) have been developed for use as an index for assessing the potential for endogenous nitrosation in man (Bartsch *et al*, 1990). The test is considered safe for use in man, since NPRO is noncarcinogenic (Garcia and Lijinsky, 1973; Nixon *et al*, 1975) and quantitively excreted in rats (Chu and Magee, 1981; Ohshima *et al*, 1982).

The NPRO test in its various forms is not without some inherent weaknesses, in particular the relatively narrow pH range of chemical acid-catalysed

nitrosation of proline (Shuker and Forman, 1991), the nitrosation of which proceeds maximally at pH 2.5 (Mirvish *et al*, 1973), a somewhat lower value than for most amines which show maximal rates at pH 3.0–3.4 (Mirvish, 1975). Other factors which may vary between individuals and affect endogenous NPRO formation include salivary nitrate recirculation (Spiegelhalder *et al*, 1976), nitrate reduction to nitrite by the oral microflora (Shapiro *et al*, 1991) and the availability of gastric nitrosation catalysts such as thiocyanate (Ruddell *et al*, 1977). Regardless of these limitations, the NPRO test has been extensively used to evaluate the potential for increased endogenous nitrosation in the gastric cancer hypothesis, population studies, and in smokers compared with nonsmokers.

The gastric cancer hypothesis

It has been hypothesized that gastric achlorhydria (pH > 4) present in some precancerous conditions such as pernicious anaemia, chronic atrophic gastritis (CAG) and gastric operations (Billroth I and Billroth II partial gastrectomies, truncal vagotomy and proximal-gastral vagotomy), could lead to proliferation of nitrate-reducing bacteria and a subsequent increase in endogenous nitrosation (Correa *et al*, 1975). This hypothesis is supported by positive correlations between fasting gastric juice pH and nitrite levels (Tannenbaum *et al*, 1979; Schlag *et al*, 1980; Kyrtopoulos *et al*, 1985a; Hall *et al*, 1987a; Carboni *et al*, 1988; Knight *et al*, 1991; Pignatelli *et al*, 1987, 1994) and total viable bacterial counts (Kyrtopoulos *et al*, 1985a; Crespi *et al*, 1987; Hall *et al*, 1987; Pignatelli *et al*, 1987; Carboni *et al*, 1988; O'Donnell *et al*, 1988), and the significantly reduced ($P < 0.005$) levels of gastric ascorbic acid in achlorhydric subjects with precancerous gastric pathology (O'Conner *et al*, 1989). Cellular atrophy, loss of gastric glands and their acid-secreting parietal cells and their gradual replacement by intestinal-type glands (intestinal metaplasia) in CAG begins as a multifocal lesion which rapidly spreads to cover much of the gastric mucosa, thus resulting in an increase in gastric pH (to pH 3.0–6.0, depending on the severity of the lesions). Nitrosation within the achlorhydric regions probably results through a non-acid bacterially mediated pathway in which bacterial reduction of nitrate increases the availability of nitrite which can migrate to adjacent areas with normally functioning mucosa where acid-catalysed nitrosation occurs (Leach *et al*, 1987). Alternatively, immunostimulation of the gastric epithelium in response to chronic *Helicobacter pylori* infection may increase endogenous gastric formation of NO (Shapiro and Hotchkiss, 1996).

Contrary to the gastric cancer hypothesis, the NPRO test has yielded consistent results showing that achlorhydric patient groups have a reduced potential for endogenous NPRO formation when compared with control groups with normal gastric pathology (Table 4). In subjects with severe CAG, intestinal metaplasia or dysplasia, NPRO excretion was significantly reduced ($P < 0.05$) compared with that in subjects with less advanced lesions (Crespi *et al*, 1987). Only in one study was a significant increase ($P < 0.03$) in NPRO excretion observed in achlorhydric patients after administration of proline without additional nitrate (Houghton *et al*, 1989); however, this observation was not confirmed in another study (Xu *et al*, 1993). In subjects monitored for 24 h intragastric pH, NPRO formation was significantly reduced ($P < 0.01$) in achlorhydric subjects (pH > 4.0 > 50% of 24 h) compared with subjects with acidic gastric conditions (pH < 4.0); NPRO excretion was negatively associated with the mean intragastric pH (Hall *et al*, 1987b).

Population studies

Similar to studies on achlorhydric patient collectives (Table 4), studies on populations at risk of gastric cancer have also produced inconsistent and contradictory results (Table 5). In all population studies the NPRO test has been performed without additional nitrate supplementation (Table 5; test condition 1). In some studies the inhibition of endogenous NPRO formation was also studied by co-administration of ascorbic acid (Table 5; test condition 2).

In Japanese subjects from low- and high-prevalence areas for gastric cancer, basal NPRO excretion was not different in undosed subjects; however, after proline administration it increased significantly ($P < 0.001$) only in subjects from the high-prevalence area (Kamiyama *et al*, 1987). Intake of ascorbic acid inhibited the increase in NPRO excretion only in the high-prevalence population. Since nitrate excretion in the absence of proline administration was significantly higher ($P < 0.07$) in the low-prevalence population, the potential for intragastric nitrosation appears to be considerably higher in subjects living in the high-prevalence area for gastric cancer. In Poland no difference was found between basal NPRO and nitrate excretion in low- and high-prevalence populations for gastric cancer; however, NPRO excretion after proline administration increased more in the high-prevalence population (Zatonski *et al*, 1989). In Costa Rica endogenous

Table 4. Evaluation of the gastric cancer hypothesis using the *N*-nitrosoproline test

Patient group	*n*	Test condition (mg)[a]		Urinary excretion of *N*-nitrosoproline (μg/day)				*P*[b]	Reference
		Nitrate	L-Proline	Range	Mean ± SD	Median	95% CI		
Control	9	380	500	–	180 ± 72	–	–	–	Hall *et al.* 1987b
Pernicious anaemia	10	380	500	–	1.1 ± 0.8	–	–	< 0.001	
Polygastrectomy	10	380	500	–	3.2 ± 2.3	–	–	NS	
Control (pH < 4.0)	10	380	500	–	17.9 ± 6.6	–	–	–	
Achlorhydria (pH > 4.0)	13	380	500	–	0.9 ± 0.6	–	–	< 0.001	
Control	18	300	500	–	–	3.7	1.8–7.9	–	Knight *et al.* 1991
Superficial gastritis	8	300	500	–		1.6	0.5–5.0	NS	
CAG	21	300	500	–	–	3.4	1.7–6.8	NS	
Gastric cancer	6	300	500	–		3.0	0.1–10.8	NS	
Control (pH < 4.0)	22	300	500	–		4.1	2.1–8.0	–	
Achlorhydria (pH > 4.0)	31	300	500	–		2.5	1.5–4.5	NS	
Control superficial gastritis (pH < 4.0)	12	260	500			15.8	10.3–26.0	–	Crespi *et al.* 1987
Mild CAG (pH < 4.0)	12	260	500	–	–	17.3	9.5–27.9	NS	
Severe CAG (pH < 4.0)	11	260	500	–	–	9.0	4.2–21.5	NS	
Control superficial gastritis (pH > 8.0)	15	260	500	–	–	14.4	6.4–17.9	–	
Mild CAG (pH < 8.0)	17	260	500	–	–	12.1	4.5–20.4	NS	
Severe CAG (pH > 8.0)	18	260	500	–	–	6.3	2.9–9.3	< 0.05	
Control	16	218	500	1.7–11.8		2.9	–	–	Houghton *et al.* 1989
Vagotomy	14	218	500	0.6–22.3	–	3.7	–	NS	
Partial gastrectomy	12	218	500	0.2–14.5		2.1		NS	
Pernicious anaemia	9	218	500	1.3–8.1	–	3.8	–	NS	
Control	22	200	500	ND–33.5	8.5 ± 7.8		–	–	Adam *et al.* 1989
Billroth I	5	200	500	ND	–	–	–	< 0.05	
Billroth II	26	200	500	ND–9.3	1.2 ± 2.7	–	–	< 0.05	
Proximal vagotomy	6	200	500	ND–22.9	10.5 ± 8.1		–	NS	
Control	20	–	500	ND–4.86	–	0.93	–	–	Houghton *et al.* 1989
Vagotomy	9	–	500	1.1–9.1	–	1.75	–	0.03	
Partial vagotomy	15	–	500	0.29–9.18	–	2.62	–	0.003	
Pernicious anaemia	11	–	500	ND–9.15		1.44	–	NS	
Control	29	–	300		9.27 ± 7.38		–	–	
Intestinal metaplasia	31	–	300	–	8.02 ± 5.24	–	–	NS	Xu *et al.* 1993
Gastric cancer or dysplasia	26	–	300	–	7.77 ± 6.19	–	–	NS	

Abbreviations: NPRO, *N*-nitrosoproline; CAG, chronic atropic gastritis; ND, not detected.
[a]Refer to text for NPRO test conditions.
[b]All tests of significance compare control group with different gastric pathology groups.

Table 5. Evaluation of endogenous nitrosation potential in epidemiological studies using the *N*-nitrosoproline test

Population and comment	*n*	Test conditions (mg)		Urinary excretion of nitrate (mg) and *N*-nitrosoproline (μg/day)						Reference
				Background excretion		Test condition 1[a]		Test condition 2[b]		
		L-Proline	Ascorbic acid	Nitrate	NPRO	Nitrate	NPRO	Nitrate	NPRO	
Costa Rica										
(12 h excretion; children)										
Low gastric cancer prevalence	25	1 × 500	1 × 200	–	–	12.4 [9.9–25.4]	0.54 [0.36–0.86]	14.3 [11.2–25.4]	0.28 [0.20–0.36]	Sierra *et al.* 1993
High gastric cancer prevalence	26	1 × 500	1 × 200	–	–	14.3 [9.3–27.3]	0.84* [0.51–1.67]	18.0 [12.4–32.9]	0.66* [0.31–0.98]	
Italy										
(12 h excretion, adults)										
Low gastic cancer prevalence	40	1 × 500	–	33.7 [27.0–42.0]	0.58 [0.4–0.84]	34.4 [28.8–41.0]	1.44 [1.1–1.87]	–	–	Knight *et al.* 1992
High gastric cancer prevalence	40	1 × 500	–	37.1 [29.7–46.3]	0.94* [0.7–1.24]	36.0 [29.7–43.6]	1.59 [1.2–2.08]	–	–	
Poland										
(24 h excretion; adults)										
Low gastric cancer prevalence	47	3 × 100	3 × 100	90 [7–344]	2.0 [0.4–19.3]	76 [9–239]	2.4 [0.1–18.9]	70 [22–377]	1.8 [0.1–8.9]	Zatonski *et al.* 1989
High gastric cancer prevalence	50	3 × 100	3 × 100	96 [36–475]	1.8 [ND–23.4]	118** [29–354]	2.8 [0.1–10.9]	104* [16–428]	1.2 [1.7–2.9]	
Japan										
(24 h excretion; adults)										
Low gastric cancer prevalence	52	3 × 100	3 × 100	145 [118–170]	6.1 [4.1–8.1]	117 [136–219]	7.1 [6.6–9.6]	88 [61–115]	4.9 [3.3–6.5]	Kamiyama *et al.* 1987
High gastric cancer prevalence	52	3 × 100	3 × 100	95*** [73–117]	3.8 [2.6–5.0]	116 [83–150]	12.6*** [8.5–16.7]	130 [107–160]	3.2* [2.4–4.0]	
China										
(24 h excretion; adults)										
Low oesophageal cancer prevalence	40	3 × 100	3 × 100	48 [37–61]	1.7 [0.9–2.9]	57 [38–77]	4.4 [3.3–6.4]	–	–	Lu *et al.* 1986
High oesophageal cancer prevalence	40	3 × 100	3 × 100	94*** [69–119]	5.7*** [4.1–7.7]	85*** [66–114]	8.3*** [6.4–12.3]	89 [58–115]	2.4 [1.7–2.9]	
Low oesophageal cancer prevalence	44	3 × 100	3 × 100	–	2.0 ± 2.6	–	3.4 ± 4.5	–	3.1 ± 4.4	Lu *et al.* 1987

Table 5 (continued)

Population and comment	*n*	Test conditions (mg)		Urinary excretion of nitrate (mg) and *N*-nitrosoproline (μg/day)						Reference
				Background excretion		Test condition 1[a]		Test condition 2[b]		
		L-Proline	Ascorbic acid	Nitrate	NPRO	Nitrate	NPRO	Nitrate	NPRO	
High oesophageal cancer prevalence	44	3 × 100	3 × 100	–	5.7 ± 8.2	–	10.2 ± 20.7	–	3.6 ± 5.9	
	47	3 × 100	3 × 100	–	5.9 ± 9.2	–	12.2 ± 22.7	–	4.1 ± 4.7	
	51	3 × 100	3 × 100	–	4.2 ± 6.3	–	7.0 ± 11.8	–	2.8 ± 3.1	
	57	3 × 100	3 × 100	–	6.6 ± 11.2	–	11.3 ± 24.9	–	3.5 ± 6.8	
	47	3 × 100	3 × 100	–	4.4 ± 6.2	–	7.3 ± 8.3	–	4.6 ± 4.6	
China										
(12 h excretion: adults)										
Low nasopharyngeal cancer prevalence	40	1 × 500	1 × 300	38 ± 6	3.3 ± 0.8	24 ± 5	3.2 ± 1.2	28 ± 4	2.4 ± 0.8	Zeng *et al.* 1993
High nasopharyngeal cancer prevalence	37	1 × 500	1 × 300	54 ± 9*	3.9 ± 0.9	125 ± 19***	7.6 ± 2.9	70 ± 15***	3.4 ± 0.8	
26 Counties study	1035	1 × 500	1 × 200	–	–	260 ± 130 (90–490)	12.4 ± 10.0 (2.9–45.3)	–	6.35 ± 7.75 (0.9–39.6)	Chen *et al.* 1987
69 Counties study	4000	1 × 500	1 × 200	–	–	170 ± 70 (50–360)	13.8 ± 11.9 (1.1–69.0)	–	5.08 ± 3.86 (0.6–19.4)	Wu *et al.* 1993
Thailand										
(12 h excretion: adults)										
Low cholangiocarcinoma cancer prevalence	18	1 × 500	1 × 200	–	–	57.7 ± 55.8	9.0 ± 14.8	35.3 ± 27.9	1.3 ± 2.0	Haswell-Elkins *et al.* 1994
Intermediate cholangiocarcinoma prevalence	20	1 × 500	1 × 200	–	–	63.9 ± 47.7	6.3 ± 8.3	58.3 ± 37.2	5.3 ± 10.3	
High cholangiocarcinoma	20	1 × 500	1 × 200	–	–	53.3 ± 49.0	4.2 ± 3.5	4.6 ± 36.0	11.5 ± 20.2	
cancer prevalence	21	1 × 500	1 × 200	–	–	70.2 ± 35.3	2.9 ± 2.5	98.6 ± 71.9	1.8 ± 1.4	
	20	1 × 500	1 × 200	–	–	52.7 ± 54.6	14.2 ± 19.4	62.6 ± 42.8	3.0 ± 2.1	
Seronegative for *O. viverrini* antibody	18	1 × 500	1 × 200	–	–	62.6 ± 56.4	3.5 ± 3.2	97.3 ± 71.3	2.5 ± 1.6	
Seropositive for *O. viverrini* antibody	23	1 × 500	1 × 200	–	–	60.8 ± 29.8	12.3 ± 18.7**[c]	68.2 ± 50.2	2.4 ± 2.0	

Abbreviations: NPRO, *N*-Nitrosoproline; ND, not detected.
Data reported as either arithmetic means ± SD (range) or geometric means [95% confidence limits]
All tests of significance compare areas of low- and high-prevalence for specific cancers unless otherwise stated: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[a]Test condition 1: administration of L-proline
[b]Test condition 2: administration of L-proline plus ascorbic acid.
[c]Compares seronegative against seropositive subjects.

Table 6. Evaluation of endogenous nitrosation potential in nonsmokers and smokers by using the *N*-nitrosoproline test

Experimental conditions and administration of test compounds (mg per administration)				NPRO excretion (μg/day)							
				Nonsmokers			Smokers				
Controlled diet	Nitrate	L-Proline	Ascorbic acid	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*P*[a]	Reference
Yes	–	–	–	13	3.55 ± 2.13	0.9–9.3	13	5.90 ± 4.36	1.5–14.6	< 0.05	Hoffmann and Brunnemann, 1983
Yes	–	300	–	14	3.61 ± 1.63	1.4–5.9	14	11.7 ± 15.5	0.7–49.3	< 0.05	
Yes	–	300	250	13	4.68 ± 2.51	ND–9.2	13	4.56 ± 3.73	ND–12.5	NS	
Yes	–	–	250	9	3.97 ± 3.48	0.6–12.4	8	5.96 ± 5.29	1.0–15.2	NS	
Yes	+	–	–	12	1.7 ± 0.4	ND–4.3	16	2.1 ± 0.5	ND–6.7	NS	Ladd *et al.* 1984
Yes	–	500	–	9	1.9 ± 0.7	ND–5.8	10	2.4 ± 0.6	ND–4.6	NS	
Yes	325	500		12	16.8 ± 3.6	6.4–47.6	16	42.6 ± 7.8	7.4–130.6	< 0.05	
No	–	–	–	4	0.32 ± 0.14	–	6	0.81 ± 0.51	–	NS	Nair *et al.* 1986
No	–	3 × 100	–	4	0.55 ± 0.12	–	6	2.84 ± 1.27		NS	
No	–	500	–	10	1.6 ± 0.6	ND–5.1	10	6.1 ± 2.1	ND–19.6	< 0.05	Scherer and Adlkofer, 1986

Abbreviations: NPRO, *N*-nitrosoproline; ND, not detected.
[a]Significant difference between nonsmokers and smokers.

NPRO formation was significantly higher in children living in a high-prevalence area for gastric cancer than in those living in a low-prevalence area (Sierra *et al*, 1993). NPRO excretion on the day of proline administration correlated significantly ($P < 0.001$) with the urinary nitrate level, but not when ascorbic acid was ingested. In Italy basal NPRO and nitrate excretion was higher in the high-prevalence population; however, contrary to the above studies, a reduced potential for endogenous NPRO formation was found after administration of proline (Knight *et al*, 1992). In Chinese subjects, no clear relationship was found between NPRO excretion and gastric mortality (Chen *et al*, 1987).

Oesophageal cancer mortality rates in China are positively and significantly associated with NPRO excretion after both administration of proline ($P < 0.01$) and proline plus ascorbic acid ($P < 0.01$) (Wu *et al*, 1993). Significantly higher NPRO ($P = 0.001$) and nitrate ($P = 0.001$) excretion was observed in a high-prevalence area, both prior to and after administration of proline, compared with a low-prevalence area; however, after administration of proline, the increase in NPRO excretion was more significant in the low-prevalence ($P = 0.001$) compared with the high-prevalence ($P = 0.02$) population (Lu *et al*, 1986). Contrary to this, the same authors have also reported that the increase in NPRO excretion after proline administration was significantly higher in several high-prevalence populations compared with a low-prevalence population (Lu *et al*, 1987).

In low- and high-prevalence areas for nasopharyngeal cancer in China, no significant difference was observed in basal excretion of NPRO; however, NPRO excretion after administration of proline was significantly higher ($P = 0.09$) in the high-prevalence population (Zeng *et al*, 1993). After correlation for urinary creatinine, the difference was not significant.

In contrast to the reported elevated basal excretion of NPRO and nitrate in subjects with *O. viverrini* infection, a risk factor for cholangiocarcinoma in Thailand (Srianujata *et al*, 1987), no clear increase in endogenous NPRO formation was observed after administration of proline and proline plus ascorbic acid to subjects living in five areas of contrasting incidence of cholangiocarcinoma (Srivatanakul *et al*, 1991). When subjects from high-prevalence areas were classified as either seronegative or seropositive for *O. viverrini* antibody and results corrected for urinary creatinine, no significant differences were observed.

Smokers and nonsmokers

Four studies have used the NPRO test to evaluate specifically whether smokers have an increased potential for endogenous nitrosation compared with nonsmokers (Table 6). Under experimentally controlled dietary conditions, single bolus administration of proline (300 mg) results in significantly higher ($P < 0.05$) NPRO excretion in smokers compared with nonsmokers, formation of which was totally inhibited by coadministration of ascorbic acid (250 mg) (Hoffmann and Brunnemann, 1983). Under normal dietary conditions, administration of proline after each meal (100 mg) resulted in slightly higher, but not significantly increased, excretion of NPRO in smokers (Nair *et al*, 1986). Bolus administration of proline (500 mg) under normal dietary conditions, however, resulted in a significant increase ($P < 0.05$) in NPRO excretion by smokers (Scherer and Adlkofer, 1986), but not under controlled dietary conditions (Ladd *et al*, 1984). Only when nitrate (325 mg/kg) was administered, together with proline, under controlled dietary conditions was the increase in NPRO excretion significantly higher ($P < 0.05$) in smokers (Ladd *et al*, 1984). The contradictory results obtained using different test and experimental protocols provide no clear indication that smoking is associated with an increased potential for endogenous nitrosation.

Other studies in which the influence of smoking was considered also produce contradictory results as to whether smoking increases the potential for endogenous nitrosation. In an experimental study in which subjects consumed a nutritionally normal diet but controlled for intake of nitrate, no significant effect to smoking on urinary NPRO excretion was observed after administration of proline (500 mg) and ingestion of a high-nitrate salad (168 mg NO_3), with and without additional vitamin C (325 mg) supplementation (Knight and Forman, 1987). No effect of smoking was found in a Chinese population at risk for oesophageal cancer (Wu *et al*, 1993). Smoking appeared to increase the potential for endogenous NPRO formation in patients with achlorhydria (Crespi *et al*, 1987; Knight *et al*, 1991); however, in populations living in high-prevalence areas for gastric cancer identical test conditions resulted in increased NPRO excretion in Japanese smokers (Kamiyama *et al*, 1987), but not in Polish smokers (Zatonski *et al*, 1989).

Apparent total *N*-nitroso compounds

The determination of individual *N*-nitrosamines in body fluids provides only a partial assessment of the

Table 7. Apparent total *N*-nitroso compounds (ATNC) in gastric juice as a function of pH[a]

Patient group	*n*	pH	ATNC (μmol/l) Mean ± SD	Range	Reference
Patients before and after	11	1.0–1.5	0.68 ± 0.48	0.10–1.69	Pignatelli *et al*, 1987
operations for duodenal	25	1.6–2.3	0.46 ± 0.74	ND–3.60	
ulcers or with chronic	10	2.5–3.3	0.38 ± 0.68	ND–2.25	
atropic gastritis	4	3.6–5.0	1.41 ± 0.92	0.06–2.13	
	11	5.7–7.0	1.28 ± 1.71	0.05–6.00	
	12	7.2–8.9	0.46 ± 0.59	ND–2.00	
Gall bladder	15	1.3–2.0	0.39 ± 0.77	ND–3.10	Kyrtopoulos *et al*, 1991
	7	2.2–3.8	2.05 ± 2.69	0.10–6.20	
	6	4.6–7.6	0.55 ± 0.55	ND–1.30	
Normal	30	1.1–3.5	0.41	0.01–2.29	Pignatelli *et al*, 1991
Achlorhydric	12	4.5–8.5	0.18	ND–0.50	

Abbreviations: ATNC, apparent total *N*-nitroso compounds; ND, not detected.
[a]All studies performed using the Pignatelli method to determine ATNC (Pignatelli *et al*, 1987)

extent of *N*-nitrosamine exposure because currently available methods are limited to determining known metabolically stable compounds and/or their metabolites. Group selective determination by chemical denitrosation and detection of released NO provides an estimate of the total concentration of all *N*-nitroso compounds present within a biological matrix, but provides no information about the identities or levels of individual species (Massey, 1988). However, information may be provided for the presence of *N*-nitroso compounds for which analysis procedures have not been established, provided care is taken to avoid both false-positive and false-negative interferences (Pignatelli and Walters, 1996). For this reason and the fact that the majority of the released NO stems from unknown *N*-nitroso compounds, the term 'apparent total *N*-nitroso compounds' (ATNC) is used.

Gastric juice

The analysis of ATNC in gastric juice is subject to some controversy, primarily because different analytical techniques give significantly different results. Using the Walters method in which ATNC are extracted into ethyl acetate prior to determination (Walters *et al*, 1978a), a positive relationship is found between fasting gastric juice pH and ATNC (Reed *et al*, 1981a,b; Stockbrugger *et al*, 1982; Sharma *et al*, 1984). According to these studies, fasting gastric juice of normal acidity (pH < 4.0) contains 0.1–0.3 μmol/l ATNC while achlorhydria is associated with an increase to about 1.0–1.5 μmol/l. These data are in agreement with the gastric cancer hypothesis (Correa *et al*, 1975); however, the increase in gastric ATNC in achlorhydric patients was not always observed (Kyrtopoulos *et al*, 1985a). Using the method of Bavin *et al* (1982), which does not involve prior extraction, no relationship was observed between gastric juice pH and ATNC (Milton-Thompson *et al*, 1982; Keighley *et al*, 1984; Hall *et al*, 1986). In these studies, samples of gastric juice were obtained frequently following consumption of a meal rather than fasting gastric juice, and the results obtained are approximately 5-fold higher than those using the Walters method.

In a comparison of the methods of Walters and Bavin using 146 fasting gastric juice samples (Dang Vu *et al*, 1994), a significant positive correlation ($R = 0.626$, $P < 0.01$) was found between gastric juice pH and nitrite concentrations; ATNC determined by the Walters method gave a weak positive correlation ($R = 0.206$, $P < 0.02$) with gastric pH; however, not when determined using a slight modification of the Bavin method (Dang Vu *et al*, 1983). Only a small fraction of gastric ATNC could be extracted into ethyl acetate (9.1% at pH < 4.0 and 22.1% at pH ≥ 4.0) when using the Walters method, which may explain why the Bavin method gives approximately 5-fold higher results. However, the Dang Wu method also measures some compounds that are not *N*-nitroso compounds. Similarly, the Bavin method is also affected by false-positive responses form certain *C*-nitroso compounds and *S*-nitrothiols (Barnard *et al*, 1982). The method of Walters *et al* (1978b) is also susceptible to false-positive responses from nitrolic acids and *S*-nitrothiols, in addition to false-negative responses caused by losses of non-extracted ATNC. A photolytic denitro-

sation method for the determination of released NO from gastric juice and urine has also been reported (Janini *et al*, 1993). Using this method significantly higher ATNC concentrations ($P < 0.01$) and nitrite levels ($P < 0.01$) are reported in subjects with gastric pH > 2.4 (You *et al*, 1996). Regardless of gastric pH, ATNC concentrations were higher in women than in men (0.16 *vs* 0.11 μmol/l; $P < 0.05$). False-positive responses from inorganic nitrite and nitrate may also make a significant confounding contribution to the release of NO in all the above methods.

Several of the problems inherent to these methods appear to have been partially overcome using the method of Pignatelli *et al* (1987). Representative data obtained using this method are summarized in Table 7. No significant difference was found in gastric juice ATNC with either extreme acid or basic pH (0.6 μmol/l ATNC at pH ≤ 3.3; 0.5 μmol/l ATNC at pH ≥ 7.2), while samples at pH 3.6–7.0 showed an average concentration of 1.3 μmol/l ATNC (Pignatelli *et al*, 1994). These results (both means and ranges of ATNC) are in partial agreement with those reported using the Walters method and provide additional support for the gastric cancer hypothesis. However, achlorhydria was not always associated with increased levels of gastric ATNC (Kyrtopoulos *et al*, 1991; Pignatelli *et al*, 1991; Houben *et al*, 1996).

All five of the above methods (Walters *et al*, 1978a; Bavin *et al*, 1982; Dang Wu *et al*, 1983; Pignatelli *et al*, 1987; Janini *et al*, 1993) suffer from the limitation that they do not measure the total level of ATNC as a group. All these methods are relatively slow, require nitrite scavengers, and are unlikely to measure reactive labile compounds. Further modification of the Pignatelli method has produced a method that appears to satisfy these limitations (Xu and Reed, 1993a). Using this method confirms that two separate significantly higher ATNC peaks, one at low pH (range 1.1–2.99; mean ATNC 1.28 ± 0.15 μmol/l) and the other at high pH (range 6.0–7.9; mean ATNC 3.74 ± 0.59 μmol/l) in which nitrite concentrations are strongly related to pH ($R = 0.48$; $P < 0.01$) (Xu and Reed, 1993b). Subsequent studies (Xu and Reed, 1993c) have confirmed the linear relationship between nitrite concentrations and gastric pH ($R = 0.887$; $P < 0.01$) above pH 5 and the presence of two ATNC peaks at low pH (range 1.1–2.99; mean ATNC 1.45 ± 0.17 μmol/l; $P < 0.05$) and at high pH (range 6.0–8.4; mean ATNC 3.57 ± 0.33 μmol/l; $P < 0.01$). The data from more than 1,000 samples of fresh gastric juice have been analysed using this method, confirming the presence of two ATNC peaks, one at low pH (2–3) and the other at high pH (6–8), reflecting a significant relationship between clinical conditions associated with increased gastric cancer risk and gastric ATNC levels (Reed *et al*, 1996). Thus providing further support for the Correa hypothesis (Correa *et al*, 1975). Representative data using this method are summarized in Table 8.

The formation of ATNC during *in vitro* incubation of human gastric juice with nitrite shows first-order dependence on nitrite and a pH profile with a sharp and continuous increase in rate below pH 3.0 (Kyrtopoulos *et al*, 1985b). This indicates that gastric ATNC are predominantly formed by nitrosation of weakly basic nitrogenous compounds (Kyrtopoulos, 1989), in agreement with theoretical studies (Shephard *et al*, 1987). The total *in vivo* gastric flux of ATNC may be as high as 5 μmol/day (Kyrtopoulos *et al*, 1991), corresponding to about 720 μg/day NPRO. Enterohepatic recycling of ATNC probably accounts for the observed levels in bile (Caygill *et al*, 1991).

Faeces

Using the Walters method, large differences are found in faecal ATNC excretion which range between 0.91–13.41 and 2.27–5.68 μmol/kg ATNC in men and women, respectively (Rowland *et al*, 1991). Total daily excretion ranges between 0.07–2.27 and 0.11–0.61 μmol/day ATNC in men and women, respectively, with a 13-fold intraindividual variation (0.16–2.16 μmol/day ATNC). In an experimental study involving eight volunteers without dietary restriction, the mean excretion for each subject on three different days ranged between 0.45–5.33 μmol/day ATNC with a grand mean of 1.56 ± 1.56 μmol/day ATNC. After consumption of a low-nitrate diet (11.0–11.6 mg nitrate/day) for 8 continuous days, faecal excretion decreased to 0.28 ± 0.26 (range ND–0.75) μmol/day ATNC and increased to 1.36 ± 1.39 (range 0.13–4.22) μmol/day ATNC after supplementation of the low-nitrate diet with an oral dose of nitrate (300 mg) for 4 continuous days. Only under controlled dietary conditions with oral nitrate supplementation was a positive correlation ($R = 0.70$; $P < 0.1$) found between faecal ATNC and urinary nitrate excretion.

Using the Pignatelli method, faecal excretion was reported to be 10.56 ± 8.60 (range 1.3–33.1) μmol/kg ATNC (Pignatelli *et al*, 1990). Using this method, faecal ATNC concentrations are considerably higher than those reported in gastric juice (Table 7), since

Table 8. Nitrate and apparent total *N*-nitroso compounds (ATNC) in gastric juice as a function of pH[a]

Patient group	*n*	pH	Nitrate (μmol/l) Mean ± SE	ATNC (μmol/l) Mean ± SE	Reference
Normal	54	2.5	4.1 ± 3.0	1.6 ± 0.3	Reed *et al.* 1993
Duodenal ulcer	20	1.9	0.3 ± 0.1	1.6 ± 1.2	
Gastric ulcer	8	2.4	2.9 ± 2.5	2.5 ± 1.0	
Vagotomy and pyloroplasty	19	3.4	6.4 ± 3.9	2.2 ± 0.7	
Chronic atrophic gastritis	11	5.5	24.0 ± 11.8	2.8 ± 1.0	
Partial gastrectomy	40	6.5	19.8 ± 3.5	2.2 ± 0.4	
Pernicious anaemia	11	7.5	68.6 ± 11.7	2.8 ± 0.8	
Gastric cancer	9	4.9	144.5 ± 78.0	4.7 ± 2.1	
Normal	44	2.9	3.9 ± 1.6	1.3 ± 0.2	Reed *et al.* 1995
Duodenal ulcer	16	1.9	0.6 ± 0.6	1.2 ± 0.5	
Gastric ulcer	10	3.0	9.6 ± 9.6	1.8 ± 0.7	
Vagotomy and pyloroplasty	11	4.1	28.4 ± 15.0	1.7 ± 0.4	
Chronic atrophic gastritis	14	5.1	29.4 ± 7.5	3.0 ± 0.8	
Partial gastrectomy	9	7.5	81.4 ± 30.1	3.9 ± 0.7	
Pernicious anaemia	9	8.2	55.0 ± 5.9	3.3 ± 1.0	
Gastric cancer	3	7.7	25.0 ± 9.5	7.1 ± 0.7	

Abbreviations: ATNC, apparent total *N*-nitroso compounds.
[a]All studies performed using the improved Pignatelli method to determine ATNC (Xu and Reed, 1993).

ATNC concentrations are 10-fold higher in the large intestine than in the stomach of rats (Massey *et al.* 1988), it appears that additional endogenous formation of ATNC may also occur in the large intestine.

Urine

Using the Walters method, bacterial infection of the urinary bladder increases urinary excretion from 0.71 ± 1.13 (range 0.01–2.93) μmol/l ATNC to 1.69 ± 2.86 (range 0.03–10.81) μmol/l ATNC (Ohshima *et al.* 1987). Urinary excretion of 0.13 ± 0.11 (range ND–0.40) μmol/l ATNC in Egyptian subjects was significantly increased ($P < 0.05$) to 0.23 ± 0.18 (range 0.05–0.70) μmol/l ATNC in patients with bacteriurea and 0.20 ± 0.41 (range 0.01–1.86) μmol/l ATNC when accompanied by *S. haematobium* infection (Hicks *et al.* 1982). As discussed previously, the Walters method probably underestimates the actual ATNC concentration in urine. Similarly, inadequate analytical methodology probably accounts for the low level of 0.28 ± 0.14 (range 0.06–0.61) μmol/l ATNC reported for sterile urines in another study (Cox *et al.* 1982).

Using the Pignatelli method, healthy adults have a urinary excretion of 1.00 ± 0.81 (range 0.11–3.20) μmol/l ATNC (Pignatelli *et al.* 1989). Following administration of proline (500 mg), 12 h urine samples contained 0.82 ± 0.65 (range 0.20–2.63) μmol/l ATNC in which total excretion of NVNA (NSAR, NPRO, NTCA and NMTCA) accounted for 16.3 ± 7.7 (range 4.7–25.9)% of the ATNC response. Median urinary ATNC excretion is significantly lower in healthy subjects (0.31 μmol/day) than in patients with Kock (0.63 μmol/day; $P = 0.0001$) and hemi-Kock (0.50 μmol/day; $P = 0.0001$) urinary diversions (Pignatelli *et al.* 1996).

Using the Janini method, the presence of CAG, intestinal metaplasia and dysplasia has little effect on urinary excretion of ATNC, NPRO, NTCA and NMTCA; however, total ATNC levels were significantly higher ($P < 0.05$) in women than in men (You *et al.* 1996).

Discussion

Prior to the American National Academy of Sciences assessment of human exposure to preformed *N*-nitroso compounds in 1981 (National Academy of Sciences, 1981), only eight *N*-nitrosamines had been identified in foods and beverages compared with the 25 *N*-nitroso compounds now known to occur in the diet (Sen, 1988; Tricker and Preussmann, 1991a; Lilliard and Hotchkiss, 1994; Pensabene and Fiddler, 1994; Sen *et al.* 1995). Analytical studies show that about 15% of the ATNC response of various foods can be accounted for by currently identified *N*-nitrosamines (Tricker *et al.* 1985; Massey *et al.* 1991) and that most of the unidentified ATNC response is due to insoluble

protein-bound species (Tricker *et al*, 1985), such as NPRO present in cured meats (Sen *et al*, 1989). As a consequence, both the 1981 and subsequent assessments of a total dietary exposure to 10–100 μg/day NVNA (Hotchkiss, 1989; Tricker and Preussmann, 1991a) may still be conservative. More recent studies in the UK estimate an adult to have a total mean dietary exposure to 0.79 μmol/day ATNC (Ministry of Agriculture, Fisheries and Food, 1992). This ATNC level is equivalent to 114 μg/day NPRO, suggesting that average total daily exposure to *N*-nitrosamines in the diet is most probably in the region of 80–120 μg/day.

Data available prior to 1981 indicated that TSNA in mainstream cigarette smoke contributed 16.2 μg/day to the total smoking-related exposure of 17.0 μg/day *N*-nitrosamines (Hoffmann *et al*, 1979, 1980). Although TSNA are still quantitatively the major *N*-nitroso compounds present in cigarette smoke (Tricker *et al*, 1991b), more current data for European and North American filter cigarettes (about 95% of the commercial cigarette market) show significant reductions in TSNA levels (Fischer *et al*, 1990a,b; Tricker *et al*, 1991b). As a result, smoking 20 cigarettes/day is now estimated to result in an exposure of about 3.0 μg/day TSNA and 0.4 μg/day VNA (Tricker *et al*, 1991b). This corresponds to about 0.02 μmol/day *N*-nitroso compounds; equivalent to only 3.0% of the total daily dietary intake of ATNC.

Occupational exposure to *N*-nitrosamines was evaluated to be 10–180 μg/day in 1981 (National Academy of Sciences, 1981), and still remains the major source of exposure in most countries without occupational health and safety standards. Based on the latest NIOSH survey (Rey and Fajen, 1996), worker exposure to 5.25 ± 3.41 (range 0.76–16.3) $\mu g/m^3$ total VNA in breathing zone air samples collected in the rubber industry indicates a mean 8 h exposure to 35.0 ± 22.7 (range 5.1–109.6) μg/day total VNA. This corresponds to 0.41 ± 0.28 (range 0.06–1.32) μmol/day total VNA assuming a respiratory volume of 20 m^3/day. Under poor working conditions in the former USSR, maximum total exposure of 680 μg/day VNA (about 7 μmol/day) in some areas of the rubber industry may still occur (Sokolskaia *et al*, 1993). Under good working conditions, in which occupational health and safety standards are maintained, air monitoring indicates an exposure of <0.1 μg/kg body weight/day (about 0.08 μmol/day) VNA (Wolf, 1989). Despite improvement of working conditions in the metal working industry, urinary biomonitoring shows exposure to 20 μg/day (0.15 μmol/day) NDELA may still occur (Bolm-Audorff *et al*, 1992). Therefore, occupational exposure is estimated to be about 0.15–0.30 μmol/day, which corresponds to about 38% of the total daily dietary intake of ATNC.

Compared with the 1981 exposure estimate of 0.4 μg/day *N*-nitrosamines caused by the use of cosmetics (National Academy of Sciences, 1981), normal use of cosmetics and personal care products are currently considered to be a negligible source of *N*-nitrosamine exposure (European Chemical Industry Ecology and Toxicology Center, 1990). Similarly, other environmental sources such as pharmaceutical products, ambient and indoor air are not normally considered to present a significant source of exposure to *N*-nitrosamines. *N*-nitrosamines in ETS (Brunnemann *et al*, 1992; Klus *et al*, 1992; Tricker *et al*, 1994), could in a worst-case situation, result in a maximum total exposure of 20 ng/day TSNA. This corresponds to about 0.1 nmol/day TSNA, or 0.012% of the total daily dietary intake of ATNC.

Based on the above evaluations, total exogenous exposure to *N*-nitrosamines is estimated to be about 1.10 μmol/day; the major exposure sources are the diet (0.79 μmol/day; 80–120 μg/day; 72%), occupational exposure (0.15–0.30 μmol/day; 20–80 μg/day; 25%), cigarette smoking (0.02 μmol/day; 3.4 μg/day; 2%) and miscellaneous minor sources such as pharmaceutical products, cosmetics, indoor and outdoor air (0.001 μmol/day; 0.1 μg/day; 1%). This assessment is significantly different to that of the American National Academy of Sciences, who in 1981, assessed the main sources of exposure to be the diet (0.3–20.0 μg/day), cigarette smoking (17 μg/day), cosmetics (0.4 μg/day) and occupational exposure (10–180 μg/day) (National Academy of Sciences, 1981).

With the exception of certain NVNA, most *N*-nitrosamines to which man is exposed are only partially excreted in urine, if at all, and the extent of their endogenous formation remains unknown. The excretion of NVNA observed in some Chinese areas with a high prevalence of oesophageal cancer, although generally elevated compared with the respective areas with low prevalence rates (Lu *et al*, 1986, 1987), are no higher than reported in normal healthy subjects in (West) Germany (Tricker *et al*, 1989b), Japan (Tsuda *et al*, 1987) or the UK (Tricker, 1996). Conversely, healthy Egyptian subjects (Tricker *et al*, 1989b) have a considerably higher NVNA excretion than subjects living in high-prevalence areas for gastric cancer in Poland (Zatonski

et al, 1989) and Japan (Kamiyama *et al*, 1987), or populations with a high-risk for oesophageal cancer in Japan (Tsugane *et al*, 1992) and China (Lu *et al*, 1986, 1987). These studies reflect both regional differences in the incidence and pattern of occurrence of dietary *N*-nitrosamines (Gao *et al*, 1991) and large interindividual variations in excretion (Garland *et al*, 1986; Tannenbaum, 1986) which may not necessarily be related to cancer risk in different geographical areas.

Determining NPRO excretion after administration of proline (NPRO test) as an index of endogenous nitrosation has several inherent weaknesses, even when corrections are made for the excretion of dietary NPRO intake (Licht and Dean, 1988; Shuker and Forman, 1991). Proline is only nitrosated under a narrow acidic pH range (pH 1.0–3.0), somewhat lower than for most amines (Mirvish, 1975), is not nitrosated by bacteria (Calmels *et al*, 1985), and is a poor substrate for cell-mediated nitrosation (Miwa *et al*, 1989). As a consequence, the NPRO test shows reduced NPRO formation in achlorhydric subjects in contrast to the gastric cancer hypothesis (Correa *et al*, 1975) (Table 4). These inherent weaknesses may also explain why the NPRO test produces inconsistent and contradictory results in population studies (Table 5) and in comparisons of smokers and nonsmokers (Table 6). The NPRO test also indicates that consumption of betel with and without tobacco increases the potential for endogenous *N*-nitrosation (Nair *et al*, 1986; Chakradeo *et al*, 1994), although no conclusive evidence is available to support an increase in urinary NVNA excretion (Nair *et al*, 1985, 1986; Chakradeo *et al*, 1994). Contrary to this, chewing tobacco does not increase (and probably inhibits) *in vivo* nitrosation (Mirvish *et al*, 1995). From these studies it can only be concluded that the various NPRO test protocols (Bartsch *et al*, 1990) require further development if possible confounding factors are to be avoided and meaningful results obtained (Mirvish *et al*, 1995).

The endogenous nitrosation of pharmaceutical products containing piperazine is probably the best example currently available to show the potential for endogenous *N*-nitrosation in man (Bellander, 1990; Tricker *et al*, 1991d; Kumar *et al*, 1992). Endogenous nitrosation of a therapeutic dose of piperazine (2 g/day) can result in endogenous formation and urinary excretion of 0.6–105 μg/day (0.005–0.90 μmol/day) *N*-nitrosamines. However, the actual extent of endogenous nitrosation is probably very much higher (Bellander, 1990). Under normal conditions, high gastric concentrations of other readily nitrosatable amine precursors would also be expected to result in significant levels of endogenous *N*-nitrosamine formation.

Continuous endogenous formation of *N*-nitrosamine is evident in both experimental animals (Van Stee *et al*, 1995) and in man (Dunn *et al*, 1990). However, the full extent of bacterial and cell-mediated nitrosation is impossible to evaluate. Increased bacterial nitrosation occurs in the achlorhydric stomach (Tables 7 and 8), and in patients with infections of the urinary tract (Ohshima *et al*, 1987; Stickler *et al*, 1992) or urinary diversions (Tricker *et al*, 1989c; Gröschel *et al*, 1992). Increased cell-mediated NO production by blood mononuclear leukocytes in alcoholic cirrhosis (Criado-Jiménez *et al*, 1995) may be related to the increased excretion of NVNA (Habib *et al*, 1986; Bartsch *et al*, 1990). Inflammatory cell infiltration in the liver (Paronetto and Tennant, 1990) and immunostimulation of hepatocytes (Curran *et al*, 1989), macrophages (Marletta *et al*, 1988) and Kupffer cells (Billiar *et al*, 1989) may also contribute to the increased *N*-nitrosamine excretion in subjects in-fected by *O. viverrini* (Srianujata *et al*, 1987) and *S. mansoni* (Mostaffa *et al*, 1994). Increased endogen-ous *N*-nitrosamine formation may be a byproduct of increased NO synthesis, a general response involved in defence against infecting organisms and inflammation (Lui and Hotchkiss, 1995).

It is also speculated that bacterial nitrosation occurs in the nasopharynx (Charrière *et al*, 1991) and vaginal vault (Pavic, 1984). Similarly, *Candida albicans* and other yeasts (*C. tropicalis*, *C. parapsilosis* and *Torulopis glabrata*) may increase the potential for endogenous *N*-nitrosamine formation in the oral cavity of subjects with poor oral hygiene (Krogh *et al*, 1987). Although not extensively studied, NDMA has been reported in breast milk (Mizuishi *et al*, 1987; Ulibu *et al*, 1996) and in pooled vaginal exudate from patients with *Trichomonas vaginalis* (Harington *et al*, 1973), and NPRO in sweat (Bogovski *et al*, 1984).

Nothing is currently known about the extent of dietary ATNC absorption in the gastrointestinal tract and excretion in urine and faeces. Gastric ATNC are elevated in subjects with CAG and achlorhydria (Pignatelli *et al*, 1987, 1991; Xu and Reed, 1993b,c; Reed *et al*, 1996). Further evidence suggests that endogenous formation of ATNC also occurs in the intestine (Massey *et al*, 1988); both nitrate and nitrite are formed endogenously in the human intestine (Tannenbaum *et al*, 1978), and several strains of intestinal bacteria can catalyse

N-nitrosation (Suzuki and Mitsuoka, 1984). Total faecal ATNC excretion is reported to be 1.56 ± 1.56 (range 0.45–5.33) μmol/day ATNC (Rowland *et al*, 1991). Assuming that an adult has an excretion of 300 g faeces/day, a total faecal excretion of 3.17 ± 2.58 (range 0.39–9.93) μmol/day ATNC can be calculated from another study (Pignatelli *et al*, 1990). VNA are not present in faeces (Lee *et al*, 1981); however, the presence of NVNA cannot be excluded (Ohshima *et al*, 1982; Van Broekhoven *et al*, 1989; Tricker *et al*, 1993).

Mean urinary excretion of ATNC amounts to 1.00 ± 0.81 (range 0.2–3.2) μmol/l urine (Pignatelli *et al*, 1989). Assuming that an adult has a mean urinary excretion of 1.3 l/day, a total urinary excretion of 1.30 ± 1.05 μmol/day ATNC can be estimated. Excretion of NVNA (NSAR, NPRO, NTCA and NMTCA) accounts for about 16% of the ATNC response (Pignatelli *et al*, 1989). From other studies the contribution of NDMA (Garland *et al*, 1986) and NHPRO (Tricker and Preussmann, 1987) to the urinary ATNC response can be calculated to be about 0.03% and 0.6%, respectively. In cigarette smokers total NNAL and NNAL-Glue excretion (Table 3) would account for about 0.2% of the ATNC response, or < 1.5% of the total identified *N*-nitroso compounds in urine. In subjects with bacterial infection of the urinary bladder the excretion of urinary ATNC is increased (Hicks *et al*, 1982; Ohshima *et al*, 1987).

A crude mass balance between total exogenous exposure to *N*-nitrosamines (1.10 μmol/day) and ATNC excretion (faeces, 1.56 ± 1.56 to 3.17 ± 2.58 μmol/day; urine, 1.30 ± 1.05 μmol/day) indicates that about 40–75% of the total human exposure to *N*-nitroso compounds results from *in vivo* formation. However, the validity of this estimate is limited, since dietary intake, accounting for 72% of the total exogenous exposure, and faecal ATNC excretion were determined by the same working group using the Walters method, while urinary ATNC was calculated from data obtained using the Pignatelli method. Nevertheless, a crude mass balance based only on data obtained for ATNC using the Walters method (*ie* dietary intake and faecal excretion) indicates that *in vivo* formation of *N*-nitroso compounds accounts for at least 50% of the total human exposure. Thus, confirmation is provided that endogenous formation of *N*-nitroso compounds could provide the largest source of exposure for the general population (National Academy of Sciences, 1978).

In light of the above comments it would appear that suitable biomonitoring methods to detect human exposure to the vast majority of *N*-nitrosamines are still not available, and basic research has often focused on identifying and developing methods for determining minor sources of exposure. It still remains to be established whether exogenous exposure to preformed *N*-nitrosamines and endogenous nitrosation of secondary amines or primary amines to form DNA-reactive species (Huber and Lutz, 1984; Shuker and Bartsch, 1994) are of secondary biologic importance to reactions of endogenous NO with intracellular and extracellular macromolecules (Lui and Hotchkiss, 1995). Reactions of NO to form *S*-nitrosothiol adducts appears to inhibit the activity of thiol-containing enzymes (Laval and Wink, 1994; Wink and Laval, 1994), indicating a dual role for endogenous NO synthesis in inducing mutation: endogenous *N*-nitrosamine formation and inhibition of repair of lesions which may be induced by these compounds. Despite intensive investigation, no human cancer has been shown to be the result of exposure to preformed *N*-nitroso compounds in the diet, tobacco, tobacco smoke and other occupation sources, or to such compounds formed *in vivo*. Although several independent pieces of circumstantial evidence support the role of *N*-nitrosamines in the aetiology of some cancers, a direct link between exposure to exogenous or endogenous *N*-nitrosamines may never be possible (Mirvish, 1995).

References

Adam B, Schlag P, Friedl P, Preussmann R, Eisenbrand G (1989). Proline is not useful as a chemical probe to measure nitrosation in the gastrointestinal tract of patients with gastric disorders characterised by anacidic conditions. *Gut* **30:** 1068–75.

Adams JD, O'Mara-Adams KJ, Hoffmann D (1987). Toxic and carcinogenic agents in undiluted mainstream smoke and sidestream smoke of different types of cigarettes. *Carcinogenesis* **8:** 729–31.

Airoldi L, Magagnotti C, De Gregoiro G, Moret M, Fanelli R (1992). *In vitro* metabolism of bladder carcinogenic nitrosamines by rat liver and urothelial cells. *Chem Biol Interact* **82:** 231–40.

Akkan Z, Preussmann R, Spiegelhalder B (1991) *N*-nitrosamines in ambient air of industrial areas in Germany. *J Cancer Res Clin Oncol* **117:** S14.

Anderson LM, Koseniauskas R, Burak ES *et al* (1992). Reduced blood clearance and increased urinary excretion of *N*-nitrosodimethylamine in patas monkeys exposed to ethanol or isopropyl alcohol. *Cancer Res* **52:** 1463–8.

Änggård E (1994). Nitric oxide: mediator, murderer and medicine. *Lancet* **343:** 1199–206.

Archer MC (1989). Mechanisms of action of *N*-nitroso compounds. *Cancer Surv* **8:** 241–50.

Barnard J, Bavin PMG, Brimblecomb RW *et al* (1982). Gastric juice, nitrite and nitroso compounds. In *Nitrosamines and Human Cancer, Banbury Report 12* (ed Magee PN); Cold Spring Harbor Laboratory, pp. 369–77.

Bartsch H (1991). *N*-nitroso compounds and human cancer: where do we stand? In *Relevance to Human Cancer of N-Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H); IARC Scientific Publications No. 105, IARC, Lyon, pp. 1–9.

Bartsch H, Ohshima H, Pignatelli B (1988). Inhibitors of endogenous nitrosation. Mechanisms and implications in human cancer prevention. *Mutat Res* **202:** 307–24.

Bartsch H, Ohshima H, Shuker DEG, Pignatelli B, Calmels S (1990). Exposure of humans to endogenous *N*-nitroso compounds: implications in cancer etiology. *Mutat Res* **238:** 255–67.

Bavin PMG, Darkin DW, Viney NJ (1982). Total nitroso compounds in gastric juice. In *N-Nitroso Compounds: Occurrence and Biological Effects* (eds Bartsch H, O'Neill IK, Castegnaro M, Okada M, Davis W); IARC Scientific Publications No. 41, IARC, Lyon, pp. 337–44.

Bellander T (1990). Nitrosation of piperazine after oral intake or inhalation exposure. In *The Significance of N-Nitrosation of Drugs* (eds Eisenbrand G, Bozler G, Von Nicolai H); Gustav Fischer Verlag, Stuttgart, pp. 213–33.

Bellander T, Österdahl B-G, Hagmar L (1985). Formation of *N*-mononitrosopiperazine in the stomach and its excretion in the urine after oral intake of piperazine. *Toxicol Appl Pharmacol* **80:** 193–8.

Bellander T, Österdahl B-G, Hagmar L, Skerfving S (1988). Excretion of *N*-mononitrosopiperazine in urine in workers manufacturing piperazine. *Int Arch Occ Environ Health* **60:** 25–9.

Bhide SV, Nair UJ, Nair J, Spiegelhalder B, Preussmann R (1986). *N*-nitrosamines in the saliva of tobacco chewers or masheri users. *Food Chem Toxic* **24:** 293–7.

Billiar TR, Curran RD, Steuhr DJ *et al* (1989). An L-arginine-dependent mechanism mediates Kupffer cell inhibition of hepatocyte protein synthesis *in vitro*. *J Exp Med* **169:** 1467–72.

Bogovski PA, Rooma MA, Kann JM (1984). Studies on the excretion of endogenously formed *N*-nitrosoproline. I. Percutaneous excretion of *N*-nitrosoproline in humans. In *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (eds O'Neill IK, Von Borstel RC, Miller CT, Long J, Bartsch H); IARC Scientific Publications No. 57, IARC, Lyon, pp. 199–204.

Bolm-Audorff U, Bienfait HG, Hiltl G, Spiegelhalder B, Preussmann R (1992). Air and biomonitoring of nitrosamine exposure in the metal processing industry. In *Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin* (eds Schaecke G, Ruppe K, Vogel-Sührig Ch); Gentner, Stuttgart, pp. 149–53.

Brunnemann KD, Hoffmann D (1978). Chemical studies on tobacco smoke. LIX. Analysis of volatile nitrosamines in tobacco smoker and polluted indoor environments. In *Environmental Aspects of N-Nitroso Compounds* (eds Walker EA, Castegnaro M, Griciute L, Lyle RE, Davis W); IARC Scientific Publications No. 19, IARC, Lyon, pp. 343–56.

Brunnemann KD, Scott JC, Hoffmann D (1983). *N*-nitrosoproline, an indicator for *N*-nitrosation of amines in processed tobacco. *J Agric Food Chem* **31:** 905–9.

Brunnemann KD, Hornby AP, Strich HF (1987). Tobacco-specific nitrosamines in the saliva of Inuit snuff dippers in the Northwest Territories of Canada. *Cancer Lett* **37:** 7–16.

Brunnemann KD, Cox JE, Hoffmann D (1992). Analysis of tobacco-specific *N*-nitrosamines in indoor air. *Carcinogenesis* **13:** 2415–8.

Caldwell WS, Greene JM, Dobson GP, de Bethizy JD (1993). Intragastric nitrosation of nicotine is not a significant contributor to nitrosamine exposure. *Ann NY Acad Sci* **686:** 213–28.

Calmels S, Ohshima H, Vincent P, Gounot A-M, Bartsch H (1985). Screening of microorganisms for nitrosation catalysis at pH 7 and kinetic studies on nitrosamine formation from secondary amines by *E. coli* strains. *Carcinogenesis* **6:** 911–5.

Calmels S, Ohshima H, Rosenkranz H, McCoy E, Bartsch H (1987). Biochemical studies on the catalysis of nitrosation by bacteria. *Carcinogenesis* **8:** 1085–8.

Calmels S, Ohshima H, Bartsch H (1988). Nitrosamine formation by denitrifying and non-denitrifying bacteria: implication of nitrite reductase and nitrate reductase in nitrosation catalysis. *J Gen Microbiol* **134:** 221–6.

Calmels S, Ohshima H, Henry Y, Bartsch H (1996). Characterization of bacterial cytochrome cd_1-nitrite reductase as one enzyme responsible for catalysis of nitrosation of secondary amines. *Carcinogenesis* **17:** 533–6.

Carboni M, Guadagni S, Pistoia MA *et al* (1988). Chronic atrophic gastritis and risk of *N*-nitroso compounds carcinogenesis. *Langenbecks Arch Chir* **373:** 82–90.

Carmella SG, Akerkar S, Hecht SS (1993). Metabolites of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers' urine. *Cancer Res* **53:** 721–4.

Carmella SG, Akerkar S, Richie Jr JP, Hecht SS (1995). Intraindividual and interindividual differences in metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers' urine. *Cancer Epidemiol Biomarkers Prev* **4:** 635–42.

Caygill CPJ, Leach SA, Fernandez F *et al* (1991). Endogneous *N*-nitrosation and cancer of the biliary tract. In *Relevance to Human Cancer of N-Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H); IARC Scientific Publications No. 105, IARC, Lyon, pp. 137–8.

Chakradeo PP, Nair J, Bhide SV (1994). Endogenous formation of *N*-nitrosoproline and other *N*-nitrosamino acids in tobacco users. *Cancer Lett* **86:** 187–94.

Challis BC (1989). Chemistry and biology of nitrosated peptides. *Cancer Surv* **8:** 363–84.

Challis BC (1996). Environmental exposures to *N*-nitroso compounds and precursors: general review of methods and current status. *Eur J Cancer Prev* **5(Suppl 1):** 19–26.

Challis BC, Colling J, Cromie DDO *et al* (1995a). A screening procedure for total *N*-nitroso contaminants in personal care products: results of collaborative studies undertaken by a CTPA Working Group. *Int J Cosmet Sci* **17:** 219–31.

Challis BC, Trew DF, Guthrie WG, Roper DV (1995b). Reduction of nitrosamines in cosmetic products. *Int J Cosmet Sci* **17:** 119–31.

Chang S-K, Harrington GW, Rothstein M *et al* (1979). Accelerating effect of ascorbic acid on *N*-nitrosamine formation and nitrosation by oxyhyponitrite. *Cancer Res* **39:** 3871–4.

Chang-Claude J, Wahrendorf J, Qiu SL *et al* (1991). Epidemiological study of precursor lesions of oesophageal cancer among young persons in Huixian, China. In *Relevance of Human Cancer of* N-*Nitroso Compounds Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H): IARC Scientific Publications No. 105, IARC, Lyon, pp. 192–6.

Charrière M, Poirier S, Calmels S *et al* (1991). Microflora of the nasopharynx in Caucasian and Maghrebian subjects with and without nasopharyngeal carcinoma. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H): IARC Scientific Publication No. 105, IARC, Lyon, pp. 158–61

Chen J, Ohshima H, Yang H *et al* (1987). A correlation study on urinary excretion of *N*-nitroso compounds and cancer mortality in China: interim results. In *Relevance of* N-*Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R): IARC Scientific Publications No. 84, IARC, Lyon, pp. 503–6.

Chu C, Magee PN (1981). Metabolic fate of nitrosoproline in the rat. *Cancer Res* **41:** 3653–7.

Cooney AH, Garland WA, Rubio F *et al* (1986a). Factors influencing the urinary excretion of nitrosodimethylamine and nitrosoproline in human beings. In *Mechanisms in Tobacco Carcinogenesis*. Banbury Report No. 23 (eds Hoffmann D, Harris CC): Cold Spring Harbor Laboratory, pp. 21–32.

Cooney RV, Ross PD, Bartolini GL (1986b). Artifactural nitrosation in the lung perfusion apparatus. *Carcinogenesis* **7:** 845–6.

Cooney RV, Hatch-Pigott V, Ross PD, Ramseryer J (1992). Carcinogenic *N*-nitrosamine formation: a requirement for nitric oxide. *J Environ Sci Health* **A27:** 789–801.

Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M (1975). A model for gastric cancer epidemiology. *Lancet* **ii:** 58–60.

Coulston F, Dunne JF (1980). *The Potential Carcinogenicity of Nitrosatable Drugs*. Ablex Publishing Corp, Norwood, NJ, USA.

Cox RD, Frank CW, Nikolaisen LD, Caputo RE (1982). Screening procedure for determination of total *N*-nitroso content in urine. *Anal Chem* **54:** 253–6.

Crespi M, Ohshima H, Ramazzotti V *et al* (1987). Intragastric nitrosation and precancerous lesions of the gastrointestinal tract: testing of an etiological hypothesis. In *Relevance of* N-*Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R): IARC Scientific Publications No. 84, IARC, LYon, pp. 511–7.

Criado-Jiménez M, Rivas-Cabañero L, Martín-Oterino JA, Lopéz-Novoa JM, Sanchez-Rodriguez A (1995). Nitric oxide production by mononuclear leukocytes in alcoholic cirrhosis. *J Mol Med* **73:** 31–3.

Curren RD, Billiar TR, Steuhr DJ, Hofmann K, Simmons RL (1989). Hepatocytes produce nitrogen oxides from L-arginine in response to inflammatory products of Kupffer cells. *J Exp Med* **170:** 1769–74.

Dang Vu B, Paul JL, Ekindjian OG, Yonger J, Gaudric M, Guerre J (1983). Chemiluminometry of nitrite and *N*-nitroso compounds in gastric juice. *Clin Chem* **29:** 1860–1.

Dang Vu B, Paul JL, Gaudric M *et al* (1994). *N*-nitroso compounds, nitrite and pH in human fasting gastric juice. *Carcinogenesis* **15:** 2657–9.

Dawson BA, Lawrence RC (1987a). Analysis of selected drug formulations for volatile nitrosamines. *J AOAC Int* **80:** 554–6.

Dawson BA, Lawrence RC (1987b). Analysis of piperazine drug formulations for *N*-nitrosamines. *J AOAC Int* **80:** 840–1.

Dietrich M, Meger M, Meger-Kossien I *et al* (1996). Exposure to *N*-nitrosamines in a rubber producing factory. *Eur J Cancer Prev* **5(Suppl 1):** 156.

Djordjevic MV, Brunnemann KD, Hoffmann D (1989). Identification and analysis of a nicotine-derived *N*-nitrosamino acid and other nitrosamino acids in tobacco. *Carcinogenesis* **10:** 1725–31.

Djordjevic MV, Sigountos CW, Brunnemann KD, Hoffmann D (1991). Formation of 4-(methylnitrosamino)-4-(3-pyridyl)butyric acid *in vitro* and in mainstream cigarette smoke. *J Agric Food Chem* **39:** 209–13.

Dunn BP, Stich HF (1984). Determination of free and protein-bound *N*-nitrosoproline in nitrite-cured meat products. *Food Chem Toxic* **22:** 609–13.

Dunn SR, Simenhoff ML, Lele PS *et al* (1990). *N*-nitrosodimethylamine blood levels in patients with chronic renal failure: modulation of levels by ethanol and ascorbic acid. *J Natl Cancer Inst* **82:** 783–7.

Edwards GS, Peng M, Fine DH, Spiegelhalder B, Kann J (1979). Detection of *N*-nitrosodiethanolamine in human urine following application of a contaminated cosmetic. *Toxicol Lett* **4:** 217–22.

Eisenbrand G, Blankart M, Sommer H, Weber B (1991). *N*-nitrosodialkanolamines in cosmetics. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H): IARC Scientific Publications No. 105, IARC, Lyon, pp. 238–41.

Eisenbrand G, Fuchs A, Kochl W (1996). *N*-nitroso compounds in cosmetics, household commodities and cutting fluids. *Eur J Cancer Prev* **5(Suppl 1):** 41–6.

Ellen G, Schuller PL (1984). *N*-nitrosoproline in urine from patients and healthy volunteers after administration of large amounts of nitrate. In N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (eds O'Neill IK, Von Borstel RC, Miller CT, Long J, Bartsch H): IARC Scientific Publications No. 57, IARC, Lyon, pp. 193–8.

Ender F, Havre G, Helgebostad A, Koppang N, Madsen R, Cen L (1964). Isolation and identification of a hepatotoxic factor in herring meal produced from sodium nitrite preserved herring. *Naturwissenschaften* **51:** 637.

European Chemical Industrial Ecology and Toxicology Centre (1990). *Human Exposure to* N-*Nitrosamines, their Effects and a Risk Assessment for* N-*Nitroso-*

diethanolamine in Personal Care Products. ECETOC Technical Report No. 41 (European Chemical Industry Ecology and Toxicology Centre, Brussels).

Fadlallah S, Cooper SF, Perrault G, Truchon G, Lesage J (1996). *N*-nitroso compounds in the ambient air of metal factories using metal-working fluids. *Bull Environ Contam Toxicol* **57:** 867–74.

Fan TY, Morrison J, Rounbehler DP, Ross R, Fine DH, Miles W, Sen NP (1977). *N*-nitrosodiethanolamine in synthetic cutting fluids: a part-per-hundred impurity. *Science* **197:** 70–1.

Fiedler H, Wood JL (1956). Specificity studies on the β-mercaptopyruvate–cyanide transsulfuration system. *J Biol Chem* **222:** 387–97.

Fine T-Y, Tannenbaum SR (1973). Factors influencing the rate of formation of nitrosomorpholine from morpholine and nitrite: acceleration by thiocyanate and other anions. *J Agric Food Chem* **21:** 843–5.

Fischer S, Castonguay A, Kaiserman M, Spiegelhalder B, Preussmann R (1990a). Tobacco-specific nitrosamines in Canadian cigarettes. *J Cancer Res Clin Oncol* **116:** 563–8.

Fischer S, Spiegelhalder B, Preussmann R (1990b). Tobacco-specific nitrosamines in European and USA cigarettes. *Arch Geschwulstforsch* **60:** 169–77.

Forman D, Al-Dabbagh S, Knight T, Doll R (1988). Nitrate exposure and the carcinogenic process. *Ann NY Acad Sci* **534:** 597–603.

Förstermann U, Schmidt HHHW, Pollock JS *et al* (1991). Isoforms of nitric oxide synthase. Characterization and purification from different cell types. *Biochem Pharmacol* **42:** 1849–57.

Fuchs J, Burg J, Hengstler JG, Bolm-Audorff U, Oesch F (1995). DNA damage in mononuclear blood cells of metal workers exposed to *N*-nitrosodiethanolamine in synthetic cutting fluids. *Mutat Res* **342:** 95–102.

Gao J, Hotchkiss JH, Chen J (1991). Regional differences in *N*-nitrosamine content of traditional Chinese foods. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H); IARC Scientific Publications No. 105, IARC, Lyon, pp. 219–22.

Garcia H, Lijinsky W (1973). Studies on the tumorigenic effect in feeding of nitrosamino acids and of low doses of amines and nitrite in rats. *Z Krebsforsch* **79:** 141–4.

Garland WA, Kuenzig W, Rubio F *et al* (1986). Urinary excretion of nitrosodimethylamine and nitrosoproline in humans: interindividual and intraindividual differences and the effect to of administered ascorbic acid and α-tocopherol. *Cancer Res* **46:** 5392–400.

Gaston B, Reilly J, Drazen JM *et al* (1993). Endogenous nitrogen oxides and bronchodilator *S*-nitrosothiols in human airways. *Proc Natl Acad Sci USA* **90:** 10957–61.

Gaston B, Drazen JM, Loscalzo J, Stamler JS (1994). The biology of nitric oxide in the airways. *Am J Respir Crit Care Med* **149:** 538–51.

Gillat PN, Palmer RC, Smith PLR, Walters CL, Reed PI (1985). Susceptibilities of drugs to nitrosation under stimulated gastric conditions. *Food Chem Toxic* **23:** 849–55.

Green LC, Ruiz de Luzuriaga K, Wagner DA *et al* (1981). Nitrate biosynthesis in man. *Proc Natl Acad Sci USA* **78:** 7764–8.

Grisham MB, Miles AM (1994). Effects of aminosalicylates and immunosuppressive agents on nitric oxide-dependent *N*-nitrosation reactions. *Biochem Pharmacol* **47:** 1897–902.

Grisham MB, Ware K, Gilleland Jr HE *et al* (1992). Neutrophil mediated nitrosamine formation: role of nitric oxide in rats. *Gastroenterology* **103:** 1260–6.

Grob K (1962). Gas chromatography of cigarette smoke. Part 2. Improved separation using a capillary column. *Beitr Tabakforsch* **9:** 315–23.

Gröschel J, Riedasch G, Kälble T, Tricker AR (1992). Nitrosamine excretion in patients with continent ileal reservoirs for urinary diversion. *J Urol* **147:** 1013–6.

Habib NA, Smadja C, Ohshima H, Bartsch H, Wood CB (1986). Relevance of endogenous nitroso compounds in cirrhotics and patients with hepatocellular carcinomas. *Br J Cancer* **54:** 1954.

Hall CN, Darkin D, Brimblecombe R *et al* (1986). Evaluation of the nitrosamine hypothesis of gastric carcinogenesis in precancerous conditions. *Gut* **27:** 491–8.

Hall CN, Darkin D, Viney N *et al* (1987a). Evaluation of the nitrosamine hypothesis of gastric carcinogenesis in man. In *Relevance of* N-*Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R); IARC Scientific Publications No. 84, IARC, Lyon, pp. 527–30.

Hall CN, Kirkham JS, Northfield TC (1987b). Urinary *N*-nitrosoproline excretion: a further evaluation of the nitrosamine hypothesis of gastric carcinogenesis in precancerous conditions. *Gut* **28:** 216–20.

Halling H, Österdahl B-G, Carstensen J (1989). Effect of ascorbic acid on *N*-nitrosoproline excretion in the urine of lacto-vegetarians and subjects on a free-choice diet. *Food Add Contam* **6:** 445–52.

Harington JS, Nunn JR, Irwig L (1973). Dimethylamine in the human vaginal vault. *Nature* **241:** 49–50.

Harvery DC, Chow HJ (1994). Nitrosamines in sunscreens and cosmetic products: occurrence, formation and trends. In *Nitrosamines and Related* N-*Nitroso Compounds, Chemistry and Biochemistry* (eds Loeppky RN, Michejda CJ); ACS Symposium Series 553, American Chemical Society, Washington DC, pp. 20–33.

Haswell-Elkins MR, Satarug S, Tsuda M *et al* (1994). Liver fluke infection and cholangiocarcinoma: model of endogenous nitric oxide and extra gastric nitrosation in human carcinogenesis. *Mutat Res* **305:** 241–52.

Hawksworth G, Hill MJ (1974). The *in vivo* formation of *N*-nitrosamines in the rat bladder and their subsequent absorption. *Br J Cancer* **29:** 353–8.

Hecht SS (1994). Metabolic activation and detoxification of tobacco-specific nitrosamines – a model for cancer prevention strategies. *Drug Metab Rev* **26:** 373–90.

Hecht SS, Chen CB, Ornaf RM, Jacobs E, Adams JD, Hoffmann D (1978). Reaction of nicotine and sodium nitrite: formation of nitrosamines and fragmentation of the pyrrolidine ring. *J Org Chem* **43:** 72–6.

Hecht SS, Morrison JB, Winninger JA (1982). *N*-nitroso-*N*-methyldodecylamine and *N*-nitroso-*N*-methyltetradecylamine in hair-care products. *Food Chem Toxic* **20:** 165–9.

Hecht SS, Carmella SG, Murphy SE *et al* (1993). A

tobacco-specific lung carcinogen in the urine of men exposed to cigarette smoke. *N Engl J Med* **329:** 1543–6.

Hecht SS, Chung F-L, Richie Jr JP *et al* (1995). Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers. *Cancer Epidemiol Biomarkers Prev* **4:** 877–84.

Hegesh E, Shiloah J (1982). Blood nitrates and infantile methemoglobinemia. *Clin Chim Acta* **125:** 107–15.

Helgason T, Ewen SWB, Jaffray B *et al* (1984). *N*-nitrosamines in smoked meats and their relation to diabetes. In N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (eds O'Neill IK, Von Borstel RC, Miller CT, Long J, Bartsch H); IARC Scientific Publications No. 57, IARC, Lyon, pp. 911–20.

Helser MA, Hotchkiss JH, Roe DA (1991). Temporal influence of ascorbic acid dose on the endogenous formation of *N*-nitrosoproline and *N*-nitrosothiazolidine-4-carboxylic acid in humans. *J Nutr Biochem* **2:** 268–73.

Hicks RM, Ismail MM, Walters CL *et al* (1982). Association of bacteriuria and urinary nitrosamine formation with *Schistosomiasis haematobium* infection in Qayub area of Egypt. *Trans R Soc Trop Med Hyg* **76:** 519–27.

Hill MJ (1996). *N*-nitrosation at non-gastric sites. *Eur J Cancer Prev* **5(Suppl 1):** 105–8.

Hiller FC, McCusker KT, Mazumder MK, Wilson JD, Bone RC (1982). Deposition of sidestream cigarette smoke in the human respiratory tract. *Am Rev Resp Dis* **125:** 406–8.

Hinds W, First MW, Huber GL, Shea JW (1983). A method for measuring respiratory deposition of cigarette smoke during smoking. *Am Ind Hyg Assoc J* **44:** 113–8.

Hoffmann D, Adams JD (1981). Carcinogenic tobacco-specific *N*-nitrosamines in snuff and saliva of snuff-dippers. *Cancer Res* **41:** 4305–8.

Hoffmann D, Brunnemann KD (1983). Endogenous formation of *N*-nitrosoproline in cigarette smokers. *Cancer Res* **43:** 5570–4.

Hoffmann D, Adams JD, Brunnemann KD, Hecht SS (1979). Assessment of tobacco-specific *N*-nitrosamines in tobacco products. *Cancer Res* **39:** 2505–9.

Hoffmann D, Adams JD, Piade JJ, Hecht SS (1980). Chemical studies on tobacco smoke. LXVIII. Analysis of volatile and tobacco-specific nitrosamines in tobacco products. In N-*Nitroso Compounds: Analysis, Formation and Occurrence* (eds Walker EA, Castegnaro M, Griciute L, Börzsöyi M, Davis W); IARC Scientific Publications No. 31, IARC, Lyon, pp. 507–14.

Hoffmann D, Brunnemann KD, Prokopczyk B, Djordjevic MV (1994). Tobacco-specific *N*-nitrosamines and *Areca*-derived *N*-nitrosamines: chemistry, biochemistry, carcinogenicity and relevance to humans. *J Toxicol Environ Health* **41:** 1–52.

Hotchkiss JH (1989). Preformed *N*-nitroso compounds in foods and beverages. *Cancer Surv* **8:** 295–321.

Houben GMP, Hooi J, Brummer RJM, Stobbering EE, Stockbrugger RW (1996). Are intragastric *N*-nitroso compounds elevated after short-term acid suppression. *Eur J Cancer Prev* **5(Suppl 1):** 83–7.

Houghton PWJ, Leach S, Owen RW *et al* (1989). Use of a modified *N*-nitrosoproline test to show intragastric nitrosation in patients at risk of gastric cancer. *Br J Cancer* **60:** 231–4.

Houlgate PR, Dhingra KS, Nash SL, Evans WH (1989). Determination of formaldehyde and acetaldehyde in mainstream cigarette smoke by high-performance liquid chromatography. *Analyst* **114:** 355–60.

Huber KW, Lutz WK (1984). Methylation of DNA in stomach and small intestine of rats after oral administration of methylamine and nitrite. *Carcinogenesis* **5:** 1729–32.

Ianysheva N, Litvinenko ON, Chernichenko IA, Kertes M (1991). Nitrosamine levels in the environmental air of several large cities. *Gig Sanit* **2:** 9–12.

Idris AM, Nair J, Ohshima H *et al* (1991). Unusually high levels of carcinogenic tobacco-specific nitrosamines in Sudan snuff (toombak). *Carcinogenesis* **12:** 115–8.

Idris AM, Nair J, Friesen M *et al* (1992). Carcinogenic tobacco-specific nitrosamines are present at unusually high levels in the saliva of oral snuff users in Sudan. *Carcinogenesis* **13:** 1001–5.

Iqbal ZM, Dahl K, Epstein SS (1980). Role of nitrogen dioxide in the biosynthesis of nitrosamines in mice. *Science* **207:** 1475–7.

Ischiropoulos H, Zhu L, Beckman JS (1992). Peroxynitrite formation from macrophage-derived nitric oxide. *Arch Biochem Biophys* **298:** 446–51.

Iyenger R, Stuehr DJ, Marletta MA (1987). Macrophage synthesis of nitrite, nitrate and *N*-nitrosamines: precursors and role of the respiratory burst. *Proc Natl Acad Sci USA* **84:** 6369–3.

Janini GM, Fox SD, Citre ML, Muschik GM, Issaq HJ (1993). Determination of total *N*-nitroso compounds in biological fluids by photolytic denitrosation and thermal energy analysis. *Anal Instrument* **21:** 1–9.

Ji X-B, Hollocher TC (1988). Mechanism for nitrosation of 2,3-diaminonaphthalene by *Escherichia coli*: enzymatic production of NO followed by O_2-dependent chemical nitrosation. *Appl Environ Microbiol* **54:** 1791–4.

Kälble T, Tricker AR, Hoang J *et al* (1991). Effect of vitamin C on endogenous formation of *N*-nitrosamines in ureterosigmoidostomy patients. *Urol Int* **46:** 22–6.

Kamiyama S, Ohshima H, Shimada A *et al* (1987). Urinary excretion of *N*-nitrosamino acids and nitrate by inhabitants in high- and low-risk areas for stomach cancer in Northern Japan. In *Relevance of* N-*Nitroso compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R); IARC Scientific Publications No. 84, IARC, Lyon, pp. 497–502.

Kamp E, Eisenbrand G (1991). Long-chain *N*-nitroso-*N*-methylalkylamines in commercial cosmetics, light-duty dishwashing liquids and household cleaning preparations. *Food Chem Toxic* **29:** 203–9.

Keefer LK, Roller PP (1973). *N*-nitrosation by nitrite ion in neutral and basic media. *Science* **181:** 1245–7.

Keefer LK, Goff U, Stevens J, Bennett EO (1990). Persistence of *N*-nitrosodiethanolamine contamination in American metal-working lubricants. *Food Chem Toxic* **28:** 531–4.

Keighley MRB, Youngs D, Pxon V *et al* (1984). Intragastric *N*-nitrosation is unlikely to be responsible

for gastric carcinoma developing after operations for duodenal ulcer. *Gut* **25:** 238–45.

Kelm M, Schrader J (1990). Control of coronary vascular tone by nitric oxide. *Circ Res* **66:** 1561–75.

Kenney GE, Sakr A, Lichtin JL, Chou H, Bronaught RL (1995). *In vitro* skin absorption and metabolism of Padimate-O and a nitrosamine formed in Padimate-O-containing cosmetic products. *J Soc Cosmet Chem* **46:** 117–27.

Khesina AY, Kolyadich MN, Krivosheyeva A *et al* (1996). Assessment of pollution of the Moscow city air with carcinogenic PAH and *N*-nitrosamines. *Expt Oncol* **18:** 14–8.

Klimisch H-J, Stadler L (1976). Investigation of the formation of *N*-nitrosonornicotine from nicotine-N'-oxide. *Talanta* **23:** 614–6.

Klus H, Begutter H, Scherer G, Tricker AR, Adlkofer F (1992). Tobacco-specific and volatile *N*-nitrosamines in environmental tobacco smoke of offices. *Indoor Environ* **1:** 348–50.

Knight TM, Forman D (1987). The availability of dietary nitrate for the endogenous nitrosation of L-proline. In *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R): IARC Scientific Publications No. 84, IARC, Lyon, pp. 518–23.

Knight TM, Leach S, Forman D *et al* (1991). *N*-nitrosoproline excretion in the presence and absence of gastric disease. *Eur J Cancer* **27:** 456–61.

Knight T, Pirastu R, Palli D *et al* (1992). Nitrate and *N*-nitrosoproline excretion in two Italian regions with contrasting rates of gastric cancer: the role of nitrate and other factors in endogenous nitrosation. *Int J Cancer* **50:** 736–9.

Kobayashi T, Kubota K (1980). The reaction of nitrogen dioxide with lung surface components: the reaction with cholesterol. *Chemosphere* **9:** 777–84.

Kosaka H, Wishnok JS, Miwa M, Leaf CD, Tannenbaum SR (1989). Nitrosation by stimulated macrophages. Inhibitors, enhancers and substrates. *Carcinogenesis* **10:** 563–6.

Kosaka H, Tsuda M, Kurashima Y *et al* (1990). Marked nitrosation by stimulation with lipopolysaccharide in ascorbic acid-defient rats. *Carcinogenesis* **11:** 1887–9.

Kresty LA, Carmella SG, Borukhova A *et al* (1996). Metabolites of a tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in the urine of smokeless tobacco users: relationship between urinary biomarkers and oral leukoplakia. *Cancer Epidemiol Biomarkers Prev* **5:** 521–5.

Krogh P, Hald B, Holmstrup P (1987). Possible mycological etiology of mucosal cancer: catalytic potential of infecting *Candida albicans* and other yeasts in production of *N*-nitrosobenzylamine. *Carcinogenesis* **8:** 1543–8.

Krull IS, Goff V, Silvergleid A, Fine DH (1979). *N*-nitroso compound contaminants in prescription and nonprescription drugs. *Arzneimittelforschung* **29:** 870–4.

Kumar R, Siddiqi M, Fazili Z *et al* (1992). Effect of dietary nitrate on endogenous nitrosation of piperazine in humans. *Cancer Lett* **65:** 139–43.

Kyrtopoulos SA (1989). *N*-nitroso compound formation in human gastric juice. *Cancer Surv* **8:** 423–42.

Kyrtopoulos SA, Daskalakis G, Legakis NJ *et al* (1985a). Studies in gastric carcinogenesis. II. Absence of elevated concentrations of *N*-nitroso compounds in gastric juice of Greek hypochlorhydric individuals. *Carcinogenesis* **6:** 1135–40.

Kyrtopoulos SA, Daskalakis G, Outram JR (1985b). Studies in gastric carcinogenesis. III. The kinetics of nitrosation *in vitro* and their implications for the *in vivo* formation of *N*-nitroso compounds in normal and achlorhydric populations. *Carcinogenesis* **6:** 1141–5.

Kyrtopoulos SA, Pignatelli B, Karkanias G, Golematis B, Esteve J (1991). Studies in gastric carcinogenesis. V. The effects of ascorbic acid on *N*-nitroso compound formation in human gastric juice *in vivo* and *in vitro*. *Carcinogenesis* **12:** 1371–6.

Ladd KF, Newmark HL, Archer MC (1984). *N*-nitrosation of proline in smokers and nonsmokers. *J Natl Cancer Inst* **73:** 83–7.

Lambotte-Vandepaer M, Duverger-van Bogaert M, Rollmann B (1985). Metabolism and mutagenicity of acrylonitrile: an *in vivo* study. *Environ Mutagen* **7:** 655–62.

Laval F, Wink DA (1994). Inhibition by nitric oxide of the repair protein, O^6-methylguanine-DNA-methyltransferase. *Carcinogenesis* **15:** 443–7.

Leach SA, Thompson M, Hill MJ (1987). Bacterially catalysed *N*-nitrosation reactions and their relative importance in the human stomach. *Carcinogenesis* **8:** 1907–12.

Leaf CD, Wishnok JS, Hurley JP *et al* (1990). Nitrate biosynthesis in rats, ferrets and humans. Precursor studies with L-arginine. *Carcinogenesis* **11:** 855–8.

Leaf CD, Wishnok JS, Tannenbaum SR (1991). Endogenous incorporation of nitric oxide from L-arginine into *N*-nitrosomorpholine stimulated by *Escherichia coli* lipopolysaccharide in the rat. *Carcinogenesis* **12:** 537–9.

Lee L, Archer MC, Bruce WR (1981). Absence of volatile nitrosamines in human feces. *Cancer Res* **412:** 3992–4.

Licht WR, Dean WM (1988). Theoretical model for predicting rates of nitrosamine and nitrosamide formation in the human stomach. *Carcinogenesis* **9:** 2227–37.

Licht WR, Tannenbaum SR, Deen WM (1988a). Use of ascorbic acid to inhibit nitrosation: kinetic and mass transfer considerations for an *in vitro* system. *Carcinogenesis* **9:** 365–72.

Licht WR, Fox JG, Deen WM (1988b). Effects of ascorbic acid and thiocyanate on nitrosation of proline in the dog stomach. *Carcinogenesis* **9:** 373–7.

Lijinsky W (1990). Structure activity relations in reaction of drugs with nitrite. In *The Significance of N-Nitrosation of Drugs* (eds Eisenbrand G, Bozle G, von Nicolai H): Gustav Fischer Verlag, Stuttgart, pp. 93–107.

Lijinsky W (1992). *Chemistry and Biology of N-Nitroso Compounds*. Cambridge University Press, New York.

Lillard TJ, Hotchkiss JH (1994). *N*-nitrosodiphenylamine in diphenylamine-treated apples. In *Nitrosamines and Related N-Nitroso Compounds: Chemistry and Biology* (eds Loeppky RN, Michejda CJ): ACS Symposium Series 553, American Chemical Society, Washington, DC, pp. 358–60.

Loeppky RN, Bao YT, Bae J, Yu L, Shevlin G (1994). Blocking nitrosamine formation: understanding the

chemistry of rapid nitrosation. In *Nitrosamines and Related N-Nitroso Compounds: Chemistry and Biochemistry* (eds Loeppky RN, Michejda CJ); ACS Symposium Series No. 553, American Chemical Society, Washington, DC, pp. 52–65.

Lopez-Vidriero MT, Das I, Reid LM (1977). Airway secretion: source, biochemical and theological properties. In *Respiratory Defence Mechanisms (Part 1)* (eds Brain JD, Proctor DF, Reid LM); Marcel Dekker, New York, pp. 289–356.

Lu S-H, Ohshima H, Fu H-M *et al* (1986). Urinary excretion of *N*-nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for esophageal cancer in northern China: endogenous formation of nitrosoproline and its inhibition by vitamin C. *Cancer Res* **46:** 1485–91.

Lu S-H, Yang WX, Guo LP *et al* (1987). Determination of *N*-nitrosamines in gastric juice and urine and a comparison of endogenous formation of *N*-nitrosoproline and its inhibition in subjects from high- and low-risk areas of oesophageal cancer. In *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R); IARC Scientific Publications No. 84, IARC, Lyon, pp. 538–43.

Lui RH, Hotchkiss JH (1995). Potential genotoxicity of chronically elevated nitric oxide: a review. *Mutat Res* **339:** 73–89.

Lui RH, Baldwin B, Tennant BC, Hotchkiss JH (1991). Elevated formation of nitrate and *N*-nitrosodimethylamine in woodchucks (*Marmota monax*) associated with chronic woodchuck hepatitis virus infection. *Cancer Res* **51:** 3925–9.

Mackerness CW, Leach SA, Thompson MH, Hill MJ (1989). The inhibition of bacterially mediated *N*-nitrosation by vitamin C: relevance to the inhibition of endogenous *N*-nitrosation in the achlorhydric stomach. *Carcinogenesis* **10:** 397–9.

Maeda S, Uchida S, Kisaki T (1978). Microbial degradation of nicotine-*N'*-oxide degradation products. *Agric Biol Chem* **42:** 1455–60.

Magee PN (1989). The experimental basis for the role of nitroso compounds in human cancer. *Cancer Surv* **8:** 207–39.

Magee PN, Barnes JM (1956). The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. *Br J Cancer* **10:** 114–22.

Malaveille C, Vineis P, Estéve J *et al* (1989). Levels of mutagens in the urine of smokers of black and blond tobacco correlate with their risk of bladder cancer. *Carcinogenesis* **10:** 577–86.

Marletta MA, Yoon PS, Iyengar R, Leaf CD, Wishnok JS (1988). Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. *Biochem* **27:** 8706–11.

Massey RC (1988). Analysis of *N*-nitroso compounds in foods and human body fluids. In *Nitrosamines: Toxicology and Microbiology* (ed Hill MJ); Ellis Horwood, Chichester, UK, pp. 16–47.

Massey RC, Key PE, Mallett AK, Rowland IR (1988). An investigation of the endogenous formation of apparent total *N*-nitroso compounds in conventional microflora and germ-free rats. *Food Chem Toxic* **26:** 595–600.

Massey RC, Key PE, Jones RA, Logan GL (1991). Volatile, non-volatile and total *N*-nitroso compounds in bacon. *Food Add Contam* **8:** 585–98.

Meger M, Meger-Kossien I, Dietrich M *et al* (1996). Metabolites of 4-(*N*-methylnitrosamino)-1-(3-pyridyl)-1-butanone in urine of smokers. *Eur J Cancer Res* **5(Suppl 1):** 121–4.

Mende P, Spiegelhalder B, Wacker C-D, Preussmann R (1989). Trapping of reactive intermediates from the nitrosation of primary amines by a new type of scavenger reagent. *Food Chem Toxic* **27:** 469–73.

Milligan JR, Zucker PF, Swann PF, Archer MC (1986). Alcohol consumption does not lead to urinary excretion of *N*-nitrosodimethylamine in the fasting human. *Carcinogenesis* **7:** 1401–2.

Mills AL, Alexander M (1976). *N*-nitrosamine formation by culture of several microorganisms. *Appl Environ Microbiol* **31:** 892–5.

Milton-Thompson GJ, Ahmet Z, Barnard J *et al* (1982). Intragastric acidity, bacteria, nitrite and *N*-nitroso compounds before, during and after cimetidine treatment. *Lancet* **i:** 1091–5.

Ministry of Agriculture, Fisheries and Food (1992). *Nitrate, Nitrite and N-Nitroso Compounds in Foods: Second Report. Food Surveillance Paper No. 23.* HMSO, London.

Mirvish SS (1975). Formation of *N*-nitroso compounds: chemistry, kinetics and *in vivo* occurrence. *Toxicol Appl Pharmacol* **31:** 325–51.

Mirvish SS (1995). Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in the etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* **93:** 17–48.

Mirvish SS, Sams J, Fan TY, Tannenbaum SR (1973). Kinetics of nitrosation of the amino acids proline, hydroxyproline and sarcosine. *J Natl Cancer Inst* **51:** 1833–9.

Mirvish SS, Sams JJ, Hecht SS (1977). Kinetics of nornicotine and anabasine nitrosation in relation to *N'*-nitrosonornicotine occurrence in tobacco and to tobacco-induced cancer. *J Natl Cancer Inst* **59:** 1211–3.

Mirvish SS, Issenberg P, Sams JP (1981). A study of *N*-nitrosomorpholine synthesis in rodents exposed to nitrogen dioxide and morpholine. In *N-Nitroso Compounds* (eds Scanlan RA, Tannenbaum SR); ACS Symposium Series 174, American Chemical Society, Washington DC, pp. 181–91.

Mirvish SS, Grandjean AC, Moller H *et al* (1992). *N*-nitrosoproline excretion by rural Nebraskans drinking water of varied nitrate content. *Cancer Epidemiol Biomarkers Prev* **1:** 455–61.

Mirvish SS, Grandjean AC, Reimers KJ *et al* (1995). Dosing time with ascorbic acid and nitrate, gum and tobacco chewing, fasting and other factors affecting *N*-nitrosoproline formation in healthy subjects taking proline with a standard meal. *Cancer Epidemiol Biomarkers Prev* **4:** 775–82.

Miwa M, Stuehr DJ, Marletta MA, Wishnok JS, Tannenbaum SR (1987). Nitrosation of amines by stimulated macrophates. *Carcinogenesis* **8:** 955–8.

Miwa M, Tsuda M, Kurashima Y, Hara H, Tanaka Y,

Shinohara K (1989). Macrophage-mediated *N*-nitrosation of thioproline and proline. *Biochem Biophys Res Comm* **159:** 373–8.

Mizuishi K, Takeuchi M, Yamanobe H *et al* (1987). Trace analysis of volatile *N*-nitrosamines in human milk. *Ann Rpt Tokyo Metr Res Lab Publ Health* **38:** 150–4.

Møller H, Landt J, Pedersen E *et al* (1989). Endogenous nitrosation in relation to nitrate exposure from drinking water and diet in a Danish rural population. *Cancer Res* **49:** 3117–21.

Mostaffa MH, Helmi S, Badawi AF *et al* (1994). Nitrate, nitrite and volatile *N*-nitroso compounds in urine of *Schistosoma haematobium* and *Schistosoma mansoni* infected patients. *Carcinogenesis* **15:** 619–25.

Murphy SE, Carmella SG, Idris AM, Hoffmann D (1994). Uptake and metabolism of carcinogenic levels of tobacco-specific nitrosamines by Sudanese snuff dippers. *Cancer Epidemiol Biomarkers Prev* **3:** 423–8.

Nair J, Ohshima H, Friesen M *et al* (1985). Tobacco-specific and betel nut-specific *N*-nitroso compounds: occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. *Carcinogenesis* **6:** 295–303.

Nair J, Ohshima H, Pignatelli B *et al* (1986). Modifiers of endogenous carcinogen formation: studies on *in vivo* nitrosation in tobacco users. In *Mechanisms in Tobacco Carcinogenesis* (eds Hoffmann D, Harris CC); Banbury Report 23, Cold Spring Harbor Laboratory, pp. 45–61.

National Academy of Sciences (1978). *Nitrates: An Environmental Assessment*. Coordinating Committee for Scientific and Technical Assessment of Environmental Pollutants, National Academy of Sciences, Washington, DC.

National Academy of Sciences (1981). *The Health Effects of Nitrate, Nitrite and N-Nitroso Compounds*. National Academy Press, Washington, DC.

Newmark HL, Mergens WJ (1981). α-Tocopherol (vitamin E) and its relationship to tumor induction and development. In *Inhibition of Tumor Induction and Development* (eds Zedeck MS, Lipkin M); Plenum Press, New York, pp. 127–60.

Nixon JE, Wales JH, Scanlon RA, Bills DD, Sinnhuber RO (1975). Null carcinogenic effect of large doses of nitrosoproline and nitrosohydroxyproline in Wistar rats. *Food Cosmet Toxicol* **14:** 133–5.

Norman V, Ihrig AM, Larson TM, Moss BL (1983). The effect of some nitrogenous blend components on NO/NOx and HCN levels in mainstream and sidestream smoke. *Beitr Tabakforsch Int* **12:** 55–62.

O'Connor HJ, Schorah CJ, Habibzedah N, Axon ATR, Cockel R (1989). Vitamin C in the human stomach: relation to gastric pH, gastroduodenal disease, and possible sources. *Gut* **30:** 436–42.

O'Donnell CM, Edwards C, Ware J (1988). Nitrosmine formation by clinical isolates of enteric bacteria. *FEMS Microbiol Lett* **51:** 193–8.

O'Neill IK, Ohshima H (1987). Role of cysteine as both precursor of thiazolidine 4-carboxylic acids found in human urine and possible inhibitor of endogenous nitrosation. In *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (Bartsch H, O'Neill IK, Schulte-Hermann R); IARC Scientific Publications No. 84, IARC, Lyon, pp. 322–7.

Ohshima H, Bartsch H (1981). Quantitative estimation of endogenous nitrosation in humans by monitoring *N*-nitrosoproline excreted in the urine. *Cancer Res* **41:** 3658–62.

Ohshima H, Bereziat J-C, Bartsch H (1982). Monitoring *N*-nitrosamino acids excreted in the urine and feces of rats as an index for endogenous nitrosation. *Carcinogenesis* **3:** 115–20.

Ohshima H, O'Neill IK, Friesen M, Béréziat J-C, Bartsch H (1984). Occurrence in human urine of new sulphur-containing *N*-nitrosamino acids *N*-nitrosothiazolidine 4-carboxylic acid and its 2-methyl derivative, and their formation. *J Cancer Res Clin Oncol* **108:** 121–8.

Ohshima H, Calmels S, Pignatelli B, Vincent P, Bartsch H (1987). *N*-nitrosamine formation in urinary-tract infections. In *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R); IARC Scientific Publications No. 84, IARC, Lyon, pp. 384–90.

Österdahl B-G (1990). The migration of tobacco-specific nitrosamines into the saliva of chewers of nicotine-containing chewing gum. *Food Chem Toxic* **28:** 619–22.

Österdahl B-G, Slorach S (1988). Tobacco-specific *N*-nitrosamines in the saliva of habitual male snuff dippers. *Food Add Contam* **5:** 581–6.

Palmer RMJ, Ferrige AG, Moncada S (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327:** 524–6.

Paronetto F, Tennant BC (1990). Woodchuck hepatitis virus infection: a model of human hepatic diseases and hepatocellular cancer. *Prog Liver Dis* **9:** 463–83.

Pavic N (1984). Is there a local production of nitrosamines by the vaginal microflora in anaerobic vaginosis/trichomoniasis. *Med Hypotheses* **15:** 433–6.

Pensabene JW, Fiddler W (1994). Gas chromatographic/thermal energy analyzer method for *N*-nitrosodibenzylamine in hams processed in elastic rubber netting. *J AOAC Int* **77:** 981–4.

Perciballi M, Conboy JJ, Hotchkiss JH (1989). Nitrite-cured meats as a source of endogenously and exogenously formed *N*-nitrosoproline in the ferret. *Food Chem Toxic* **27:** 111–6.

Persson MG, Wiklund NP, Gustafsson LE (1993). Endogenous nitric oxide in single exhalations and the change during exercise. *Am Rev Resp Dis* **148:** 1210–4.

Pignatelli B, Walters CL (1996). Analytical techniques for total *N*-nitroso compounds. *Eur J Cancer Prev* **5(Suppl 1):** 51–8.

Pignatelli B, Richard I, Bourgade MC, Bartsch H (1987). Improved group determination of total *N*-nitroso compounds in human gastric juice by chemical denitrosation and thermal energy analysis. *Analyst* **112:** 945–9.

Pignatelli B, Chen C-S, Thuillier P, Bartsch H (1989). Group-selective determination of total *N*-nitroso compounds in nitrate-containing human urine samples. *Analyst* **114:** 1103–8.

Pignatelli B, Chen C-S, Thuiller P, Bartsch H (1990). An improved method for the analysis of total *N*-nitroso

compounds (NOC) in biological matrices including human gastric juice. In *The Significance of* N-*Nitrosation of Drugs* (eds Eisenbrand G, Bozler G, Von Nicolai H); Gustav Fischer Verlag, Stuttgart, pp. 123–41.

Pignatelli B, Malaveille C, Chen C *et al* (1991). *N*-nitroso compounds, genotoxins and their precursors in gastric juice from humans with and without precancerous lesions of the stomach. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H); IARC Scientific Publications No. 105, IARC, Lyon, pp. 172–7.

Pignatelli B, Malaveille C, Thuillier P, Hautefeuille A, Bartsch H (1994). Improved methods for analysis of *N*-nitroso compounds and applications in human biomonitoring. In *Nitrosamines and Related Compounds: Chemistry and Biochemistry* (eds Loeppky RN, Michejda CJ); ACS Symposium Series 553, American Chemical Society, Washington, DC, pp. 102–18.

Pignatelli B, Poulsen AL, Cartensen JM *et al* (1996). Urinary total *N*-nitroso compounds in patients with urinary diversions. *Eur J Cancer Prev* **5(Suppl 1):** 103.

Pollock JRA (1985). Formation of nitrosoiminodialkanoic acids during the nitrosation of peptides. *Food Chem Toxic* **23:** 701–4.

Postlethwait EM, Mostafa MG (1983). Formation of *N*-nitrosomorpholine in isolated rat lungs during nitrogen dioxide ventilation. *Carcinogenesis* **4:** 777–8.

Preston-Martin S, Correa P (1989). Epidemiological evidence for the role of nitroso compounds in human cancer. *Cancer Surv* **8:** 459–73.

Preussmann R, Steward BW (1984). *N*-nitroso carcinogens. In *Chemical Carcinogens* 2nd Edition (ed Searle CE); ACS Monographs Series No. 182, American Chemical Society, Washington, DC, pp. 643–823.

Prokopczyk B, Wu M, Cox JE *et al* (1995). Improved methodology for the quantitative assessment of tobacco-specific *N*-nitrosamines in tobacco by supercritical fluid extraction. *J Agric Food Chem* **43:** 916–22.

Pryor WA, Lightsey JW (1981). Mechanisms of nitrogen dioxide reactions: initiation of lipid peroxidation and the production of nitrous acid. *Science* **214:** 435–7.

Ralt D, Wishnok JS, Fitts R, Tannenbaum SR (1988). Bacterial catalysis of nitrosation: involvement of the *nar* operon of *Escherichia coli*. *J Bacteriol* **170:** 359–64.

Reed PI (1996). *N*-nitroso compounds, their relevance to human cancer and further prospects for prevention. *Eur J Cancer Prev* **5(Suppl 1):** 137–47.

Reed PI, Smith PLR, Haines K, House FR, Walters CL (1981a). Gastric juice *N*-nitrosamines in health and gastroduodenal disease. *Lancet* **ii:** 550–2.

Reed PI, Smith PLR, Haines K, House FR, Walters CL (1981b). Effects of cimetidine on gastric juice *N*-nitrosamine concentration. *Lancet* **ii:** 553–6.

Reed PI, Xu GP, Li DH, Hill MJ, Johnston BJ (1993). *N*-nitroso compound levels in fresh gastric juice in relation to clinical diagnosis. *Gut* **34 (Suppl 2):** S30.

Reed PI, Shen ZZ, Jonston BJ, Hill MJ (1995). Total *N*-nitroso compound (TNNC) concentration correlates with increased risk of gastric cancer. *Gut* **37(Suppl 1):** A35.

Reed PI, Xu GP, Shen ZZ (1996). Review of studies employing an improved rapid method of measuring total *N*-nitroso compounds as a group and its use at the bedside. *Eur J Cancer Prev* **5(Suppl 1):** 89–93.

Rey BD, Fajen JM (1996). Worker exposure to nitrosamines in a rubber vehicle sealing plant. *Am Ind Hyg Assoc J* **57:** 918–23.

Ridd JH (1961). Nitrosation, diazotization and deamination. *Q Rev Chem Soc (London)* **15:** 418–41.

Ridd JH (1978). Diffusion control and pre-association in nitration and halogenation. *Adv Phy Org Chem* **16:** 1–49.

Rounbehler DP, Fajen MJ (1982). N-*Nitroso Compounds in the Factory Environment*. (Report, NIOSH Contract No. 210-77-0100). National Institute for Occupational Safety and Health, Cincinnati, OH.

Rowland IR, Granli T, Bockman OC, Key PE, Massey RC (1991). Endogenous *N*-nitrosation in man assessed by measurement of apparent total *N*-nitroso compounds in faeces. *Carcinogenesis* **12:** 1395–401.

Ruddell WSJ, Blendis LM, Walters CL (1977). Nitrite and thiocyanate in the fasting and secreting stomach and in saliva. *Gut* **18:** 73–7.

Sander J, Schweinsburg F, Menz HP (1968). Investigation on the formation of carcinogenic nitrosamines in the stomach. *Z Physiol Chem* **349:** 1691–7.

Scherer G, Adlkofer F (1986). Endogenous formation of *N*-nitrosoproline in smokers and nonsmokers. In *Mechanisms in Tobacco Carcinogenesis* (eds Hoffmann D, Harris CC); Banbury Report No. 23, Cold Spring Harbor Laboratory, pp. 137–48.

Schlag P, Böcker R, Ulrich H *et al* (1980). Are nitrite and *N*-nitroso compounds in gastric juice risk factors for carcinoma in the operated stomach. *Lancet* **i:** 727–9.

Sen NP (1988). Migration and formation of *N*-nitrosamines from food contact materials. In *Food Packaging Interactions* (ed Hotchkiss JH); ACS Symposium Series No. 365, American Chemical Society, Washington, DC, pp. 146–58.

Sen NP, Seaman SW, Miles WF (1976). Dimethylnitrosamine and nitrosopyrrolidine in fumes produced during the frying of bacon. *Food Cosmet Toxic* **14:** 167–70.

Sen NP, Baddoo PA, Seaman SW (1989). The analysis and significance of bound *N*-nitrosoproline in nitrite-cured meat products. *Food Add Contam* **6:** 21–8.

Sen NP, Baddoo PA, Weber D, Helgason T (1990). Detection of a new nitrosamine, *N*-nitroso-*N*-methylaniline, and other nitrosamines in Icelandic smoked mutton. *J Agric Food Chem* **38:** 1007–11.

Sen NP, Seaman SS, Lau PBY, Weber D, Lewis D (1995). Determination and occurrence of various tetrahydro-β-carboline-3-carboxylic acids and their corresponding *N*-nitroso compounds in foods and alcoholic beverages. *Food Chem* **54:** 327–37.

Sen NP, Seaman SW, Bergeron C, Brousseau R (1996). Trends in the levels of *N*-nitrosodimethylamine in Canadian and imported beers. *J Agric Food Chem* **44:** 1498–501.

Shapiro KB, Hotchkiss JH (1996). Induction of nitric oxide synthesis in murine macrophages by *Helicobacter pylori*. *Cancer Lett* **102:** 49–56.

Shapiro KB, Hotchkiss JH, Roe DA (1991). Quantitative relationship between oral nitrate-reducing activity and

the endogenous formation of *N*-nitrosamino acids in humans. *Food Chem Toxic* **29:** 751–5.

Sharma BK, Santana IA, Wood EC *et al* (1984). Intragastric bacterial activity and nitrosation before, during and after treatment with omeprazole. *Br Med J* **289:** 717–9.

Shephard SE, Schlatter Ch, Lutz WK (1987). Assessment of the risk of formation of carcinogenic *N*-nitroso compounds from dietary precursors in the stomach. *Food Chem Toxic* **25:** 91–108.

Shuker DEG, Forman D (1991). Practical aspects of application of dosimetric methods: summary of a workshop discussion. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H): IARC Scientific Publications No. 105, IARC, Lyon, pp. 129–34.

Shuker DEG, Bartsch H (1994). DNA adducts of nitrosamines. In *DNA Adducts: Identification and Biological Significance* (eds Hemminki K, Dipple A, Shuker DEG, Kadlubar FF, Segerbäck D, Bartsch H): IARC Scientific Publications No. 125, IARC, Lyon, pp. 73–89.

Sierra R, Chinnock A, Ohshima H *et al* (1993). *In vivo* nitrosoproline formation and other risk factors in Costa Rican children from high- and low-risk areas for gastric cancer. *Cancer Epidemiol Biomarkers Prev* **2:** 563–8.

Sipahimalani AT, Chada MS, Bhide SV, Pratap AI, Nair J (1984). Detection of *N*-nitrosamines in the saliva of habitual chewers of tobacco. *Food Chem Toxic* **22:** 261–4.

Sokolskaia NN, Krivosheeva LV, Khesing A, Piven VA, Kavun SM (1993). Carcinogenic *N*-nitrosamines in the tyre industry. *Med Tr Prom Ekol* **5:** 26–8.

Solionova LG, Smulevich VB, turbin EV, Krivosheyeva LV, Plotnikov JV (1992). Carcinogens in rubber production in the Soviet Union. *Scand J Work Environ Health* **18:** 120–3.

Spiegelhalder B (1984). Occupational exposure to *N*-nitrosamines. Air measurements and biological monitoring. In N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (eds O'Neill IK, Von Borstel RC, Miller CT, Long J, Bartsch H): IARC Scientific Publications No. 57, IARC, Lyon, pp. 937–42.

Spiegelhalder B, Preussmann R (1984). Contamination of toiletries and cosmetic products with volatile and nonvolatile *N*-nitroso carcinogens. *J Cancer Res Clin Oncol* **108:** 160–3.

Spiegelhalder B, Preussmann R (1985). *In vivo* nitrosation of amidopyrine in humans: use of 'ethanol effect' for biological monitoring of *N*-nitrosodimethylamine in urine. *Carcinogenesis* **6:** 545–8.

Spiegelhalder B, Preussmann R (1987). Nitrosamine measurements in ambient air of an industrial area in Austria. In *Relevance of* N-*Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R): IARC Science Publications No. 84, IARC, Lyon, pp. 411–4.

Spiegelhalder B, Eisenbrand G, Preussmann R (1976). Influence of dietary nitrate on nitrite content of human saliva: possible relevance to *in vivo* formation of *N*-nitroso compounds. *Food Cosmet Toxic* **14:** 545–8.

Spiegelhalder B, Eisenbrand G, Preussmann R (1982). Urinary excretion of *N*-nitrosamines in rats and humans. In N-*Nitroso Compounds: Occurrence and Biological Effects* (eds Bartsch H, O'Neill IK, Castegnaro M, Okada M, Davis W): IARC Scientific Publications No. 41, IARC, Lyon, pp. 443–9.

Spiegelhalder B, Preussmann R, Hartung M (1984). Biological monitoring in the metal working industry. In N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (eds O'Neill IK, Von Borstel RC, Miller CT, Long J, Bartsch H): IARC Scientific Publications No. 57, IARC, Lyon, pp. 943–6.

Srianujata S, Tonbuth S, Bunyaratvej S *et al* (1987). High urinary excretion of nitrate and *N*-nitrosoproline in Opisthorchiasis subjects. In *Relevance of* N-*Nitroso Compounds to Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R): IARC Scientific Publications No. 84, IARC, Lyon, pp. 544–6.

Srivatanakul P, Ohshima H, Khlat M *et al* (1991). *Opisthorchis viverrini* infestation and endogenous nitrosamines as risk factors for cholangiocarcinoma in Thailand. *Int J Cancer* **48:** 821–5.

Stamler JS, Singel DJ, Loscalzo J (1992). Biochemistry of nitric oxide and its redox-activated forms. *Science* **258:** 1898–902.

Stephany RW, Freudenthal J, Schuller PL (1978). *N*-nitroso-5-methyl-1,3-oxazolidine identified as an impurity in a commercial cutting fluid. *J Royal Neth Chem Soc* **97:** 177–8.

Stich HF, Hornby AP (1988). Endogenous and exogenous sources of nitrate/nitrite and nitrosamines in Canadian Inuit with a traditional or western lifestyle. *Circumpolar Health* **84:** 92–5.

Stich HF, Hornby AP, Dunn BP (1984). The effect of dietary factors on nitrosoproline levels in human urine. *Int J Cancer* **33:** 625–8.

Stickler DJ, Chawla JC, Tricker AR, Preussmann R (1992). *N*-nitrosamine generation by urinary tract infections in spine injured patients. *Paraplegia* **30:** 855–63.

Stillwell WG, Gogowski J, Xu H-X *et al* (1991a). Urinary excretion of nitrate, *N*-nitrosoproline, 3-methyladenine, and 7-methylguanine in a Colombian population at high risk for stomach cancer. *Cancer Res* **51:** 190–4.

Stillwell WG, Xu H-X, Glogowski J *et al* (1991b). Comparison of urinary nitrate, *N*-nitrosoproline, 7-methylguanine and 3-methyladenine levels in a human population at risk for gastric cancer. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H): IARC Scientific Publications No. 105, IARC, Lyon, pp. 83–7.

Stockbrugger RW, Cotton PB, Eugenide N *et al* (1982). Intragastric nitrites, nitrosamines and bacterial overgrowth during cimetidine treatment. *Gut* **23:** 1048–54.

Stuehr DJ, Kwon NS, Nathan CF *et al* (1991). N^{ω}-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine. *J Biol Chem* **266:** 6259–63.

Suzuki K, Mitsuoka T (1984). *N*-nitrosamine formation by intestinal bacteria. In N-*Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer* (eds O'Neill IK, Von Borsted RC, Long JE,

Miller CT, Bartsch H): IARC Scientific Publications No. 57. IARC, Lyon, pp. 275–81.

Swann PF, Coe AM, Mace R (1984). Ethanol and dimethylnitrosamine and diethylnitrosamine metabolism and deposition in the rat. Possible relevance to the influence of ethanol on human cancer incidence. *Carcinogenesis* **5:** 1337–43.

Tamir S, Lewis RS, de Rojas Walker T *et al* (1993). The influence of delivery rate on the chemistry and biological effects of nitric oxide. *Chem Res Toxicol* **6:** 895–9.

Tannenbaum SR (1986). Diet and exposure to *N*-nitroso compounds. In *Diet, Nutrition and Cancer* (ed Hayashi Y): Japan Science Society Press, Tokyo, pp. 67–75.

Tannenbaum SR, Fett D, Young VR, Land PD, Bruce WR (1978). Nitrite and nitrate are formed by endogenous synthesis in the human intestine. *Science* **200:** 1487–9.

Tannenbaum SR, Moran D, Rand W, Cuello C, Correa P (1979). Gastric cancer in Columbia. IV. Nitrite and other ions in gastric contents of residents from a high-risk region. *J Natl Cancer Inst* **62:** 9–12.

Tricker AR (1996). Excretion of *N*-nitrosamines in patients with bacterial bladder infections or diversions of the urinary tract. *Eur J Cancer Prev* **5(Suppl 1):** 95–9.

Tricker AR, Kubacki SJ (1992). Review of the occurrence and formation of non-volatile *N*-nitroso compounds in foods. *Food Add Contam* **9:** 39–69.

Tricker AR, Preussmann R (1987). Influence of cysteine and nitrite on the endogenous formation of *N*-nitrosamino acids. *Cancer Lett* **34:** 39–47.

Tricker AR, Preussmann R (1991a). Carcinogenic *N*-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. *Mutat Res* **259:** 277–89.

Tricker AR, Preussmann R (1991b). Volatile and nonvolatile nitrosamines in beer. *J Cancer Res Clin Oncol* **117:** 130–2.

Tricker AR, Preussmann R (1991c). Occurrence of an exposure to *N*-nitroso compounds in tobacco. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H): IARC Scientific Publications No. 105, IARC, Lyon, pp. 493–5.

Tricker AR, Perkins MJ, Massey RC, McWeeny DJ (1984). Synthesis of three *N*-nitroso dipeptides *N*-terminal in proline and a method for their determination in food. *Food Add Contam* **1:** 307–12.

Tricker AR, Perkins MJ, Massey RC, McWeeny DJ (1985). Some nitrosamino acids in bacon adipose tissue and their contribution to the total *N*-nitroso compound concentration. *Z Lebensm Unters Forsch* **180:** 379–83.

Tricker AR, Spiegelhalder B, Preussmann R (1989a). Environmental exposure to preformed nitroso compounds. *Cancer Surv* **8:** 251–72.

Tricker AR, Mostaffa MH, Spiegelhalder B, Preussmann R (1989b). Urinary excretion of nitrate, nitrite and *N*-nitroso compounds in *Schistosomiasis* and bilharzia bladder cancer patients. *Carcinogenesis* **10:** 547–52.

Tricker AR, Kälble T, Preussmann R (1989c). Increased urinary nitrosamine excretion in patients with urinary diversions. *Carcinogenesis* **10:** 2379–82.

Tricker AR, Pfundstein B, Theobald E, Preussmann R, Spiegelhalder B (1991a). Mean daily intake of volatile *N*-nitrosamines from foods and beverages in West Germany in 1989–1991. *Food Chem Toxic* **29:** 733–9.

Tricker AR, Ditrich C, Preussmann R (1991b). *N*-nitroso compounds in cigarette tobacco and their occurrence in mainstream tobacco smoke. *Carcinogenesis* **12:** 257–61.

Tricker AR, Stickler DJ, Chawla JC, Preussmann R (1991c). Increased urinary nitrosamine excretion in paraplegic patients. *Carcinogenesis* **12:** 943–6.

Tricker AR, Kumar R, Siddiqi M, Khuroo MS, Preussmann R (1991d). Endogenous formation of *N*-nitrosamines from piperazine and their urinary excretion following antihelmintic treatment with piperazine citrate. *Carcinogenesis* **12:** 1595–9.

Tricker AR, Kälble T, Preussmann R (1991e). Comparative metabolism and urinary excretion of *N*-mononitrosopiperazine and *N,N'*-dinitrosopiperazine in the rat. *Cancer Lett* **59:** 165–9.

Tricker AR, Pfundstein B, Kälble T, Preussmann R (1992). Secondary amine precursors to nitrosamines in human saliva, gastric juice, blood, urine and faeces. *Carcinogenesis* **13:** 563–8.

Tricker AR, Scherer G, Conze C *et al* (1993). Evaluation of 4-(*N*-methylnitrosamino)-4-(3-pyridyl)butyric acid as a potential monitor of endogenous nitrosation of nicotine and its metabolites. *Carcinogenesis* **14:** 1409–14.

Tricker AR, Klus H, Begutter H *et al* (1994). Tobacco-specific and volatile *N*-nitrosamines in indoor air of smoker and non-smoker homes. In *Indoor Air Pollution* (ed Weber L): Franz Siegel Buch GmbH, Ulm, pp. 310–6.

Tsuda M, Kurashima Y (1991a). Nitrite-trapping capacity of thioproline in the human body. In *Relevance to Human Cancer of* N-*Nitroso Compounds, Tobacco Smoke and Mycotoxins* (eds O'Neill IK, Chen J, Bartsch H): IARC Scientific Publications No. 105, IARC, Lyon, pp. 123–8.

Tsuda M, Kurashima Y (1991b). Tobacco smoking, chewing and snuff dipping: factors contributing to the endogenous formation of *N*-nitroso compounds. *Crit Rev Toxicol* **21:** 243–53.

Tsuda M, Niitsuma J, Sato S *et al* (1986). Increase in the levels of *N*-nitrosoproline, *N*-nitrosothioproline and *N*-nitroso-2-methylthioproline in human urine by cigarette smoking. *Cancer Lett* **30:** 117–24.

Tsuda M, Nagai A, Suzuki H *et al* (1987). Effect of cigarette smoking and dietary factors on the amounts of *N*-nitrosothiazolidine 4-carboxylic acid and *N*-nitroso-2-methylthiazolidine 4-carboxylic acid in human urine. In *Relevance of* N-*Nitroso Compounds on Human Cancer: Exposures and Mechanisms* (eds Bartsch H, O'Neill IK, Schulte-Hermann R): IARC Scientific Publications No. 84, IARC, Lyon, pp. 446–50.

Tsuda H, Frank N, Sato S, Sugimura T (1988). Marked increase in the urinary level of *N*-nitrosothioproline after ingestion of cod with vegetables. *Cancer Res* **48:** 4049–52.

Tsugane S, Tsuda M, Gey F, Watanabe S (1992). Cross-sectional study with multiple measurements of biological markers for assessing stomach cancer risks at the population level. *Environ Health Perspect* **98:** 207–10.

Uibu J, Tauts O, Levin A, Shimanovskaya N, Matto R (1996). *N*-nitrosodimethylamine, nitrate and nitrate-

reducing microorganisms in human milk. *Acta Paediatrica* **85:** 1140–2.

Uozumi M, Kusumoto T, Kimura T, Nakamura A, Nakajima T (1982). Formation of *N*-nitrosodimethylamine by NOx exposure in rats and rabbits pretreated with aminopyrine. In *N-Nitroso Compounds: Occurrence and Biological Effects* (eds Bartsch H, O'Neill IK, Castegnaro M, Okada M, Davies W); IARC Scientific Publications No. 41, IARC, Lyon, pp. 425–32.

Van Broekhoven LW, Davies JAR, Geurink JH (1989). The metabolism of nitrate and proline in the rumen fluid of a cow and its effect on *in vivo* formation of *N*-nitrosoproline. *Neth J Agric Sci* **37:** 157–63.

Van Stee EW, Slowane RA, Simons JE, Brunnemann KD (1983). *In vivo* formation of *N*-nitrosomorpholine in CD-1 mice exposed by inhalation to nitrogen dioxide and by gavage to morpholine. *J Natl Cancer Inst* **70:** 375–9.

Van Stee EW, Sloane RA, Simmons JE, Moorman MP, Brunnemann KD (1995). Endogenous formation of *N*-nitrosomorpholine in mice from $^{15}NO_3$ by inhalation and morpholine by gavage. *Carcinogenesis* **16:** 89–92.

Wagner DA, Shuker DEG, Bilmazes C *et al* (1985). Effect of vitamins C and E on endogenous synthesis of *N*-nitrosamino acids in humans: precursor–product studies with [^{15}N]nitrate. *Cancer Res* **45:** 6519–22.

Walters CL, Downes MJ, Edwards MW, Smith PLR (1978a). Determination of a non-volatile *N*-nitrosamine on a food matrix. *Analyst* **103:** 1127–33.

Walters CL, Hart RJ, Perse S (1978b). The breakdown into nitric oxide of compounds potentially derived from nitrite in a biological matrix. *Z Lebensm Unters Forsch* **167:** 315–9.

Webb KS, Gough TA, Carrick A, Hazelby D (1979). Mass spectrometric and chemiluminescent detection of picogram amounts of *N*-nitrosodimethylamine. *Anal Chem* **51:** 989–92.

Webb KS, Wood BJ, Gough TA (1983). The effect of the intake of a nitrosatable drug on the nitrosamine levels in human urine. *J Anal Toxicol* **7:** 181–4.

Williams DHL (1983). Nitrosation mechanisms. *Adv Phys Org Chem* **19:** 381–428.

Williams TB (1980). The determination of nitric oxide in gas phase cigarette smoke by non-dispersive infrared analysis. *Beitr Tabakforsch Int* **10:** 91–9.

Wink DA, Laval J (1994). The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide *in vitro* and *in vivo*. *Carcinogenesis* **15:** 2125–9.

Wink DA, Darbyshire JF, Nims RW, Saavedra JE, Ford PC (1993). Reactions of the bioregulatory agent nitric oxide in oxygenated aqueous media: determination of the kinetics of oxidation and nitrosation by intermediates generated by the NO/O2 reaction. *Chem Res Toxicol* **6:** 23–7.

Wolf D (1989). *N*-nitrosamine at the workplace. *Staub-Reinhalt Luft* **49:** 183–6.

Wu Y, Brouet I, Calmels S, Bartsch H, Ohshima H (1993). Increased endogenous *N*-nitrosamine and nitrate formation by induction of nitric oxide synthase in rats with acute hepatic injury caused by *Propionbacterium acnes* and lipopolysaccharide administration. *Carcinogenesis* **14:** 7–10.

Wu Y, Chen J, Ohshima H *et al* (1993). Geographic association between urinary excretion of *N*-nitroso compounds and oesophageal cancer mortality in China. *Int J Cancer* **54:** 713–9.

Xu GP, Reed PI (1993a). Method for group determination of total *N*-nitroso compounds and nitrite in fresh gastric juice by chemical denitrosation and thermal energy analysis. *Analyst* **118:** 877–83.

Xu GP, Reed PI (1993b). Instability of *N*-nitroso compounds in gastric juice and preliminary results from analysis of fresh samples by using an improved analytical method. *Eur J Cancer Prev* **2:** 381–6.

Xu GP, Reed PI (1993c). *N*-nitroso compounds in fresh gastric juice and their relation to intragastric pH and nitrite by employing an improved analytical method. *Carcinogenesis* **14:** 2547–51.

Xu GP, Song PJ, Reed PI (1993). Effects of fruit juices, processed vegetable juice, orange peel and green tea on endogenous formation of *N*-nitrosoproline in subjects from a high-risk area for gastric cancer in Moping County, China. *Eur J Cancer Prev* **2:** 327–35.

You W-C, Zhang L, Yang C-S *et al* (1996). Nitrite, *N*-nitroso compounds, and other analyses in physiological fluids in relationship to precancerous gastric lesions. *Cancer Epidemiol Biomarkers Prev* **5:** 47–52.

Zatonski W, Ohshima H, Przewozniak K *et al* (1989). Urinary excretion of *N*-nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for stomach cancer in Poland. *Int J Cancer* **44:** 823–7.

Zeng Y, Ohshima H, Bouvier G *et al* (1993). Urinary excretion of nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for nasopharyngeal carcinoma in southern China. *Cancer Epidemiol Biomarkers Prev* **2:** 195–200.

Ziebarth D (1994). *In vivo* nitrosation of metamizol in man. *Arch Pharm* **327:** 381–4.

Zumft WG (1993). The biological role of nitric oxide in bacteria. *Arch Microbiol* **160:** 253–64.